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(54) Title: METHOD AND APPARATUS FOR EFFICIENT MIMOTOPE DISCOVERY

(57) Abstract

A method for elucidating which oligomers, such as peptides, out of a plurality of synthesized oligomers is likely to be responsible for a known activity. The method involves first selecting a set of monomers suspected of being present in the oligomers to be elucidated. These monomers are grouped and mixed according to similar properties, with each monomer being represented in only one monomer grouping. The sets of monomer groupings are then divided into a first pair of binary groupings and oligomer mixtures are formed based on the binary groupings, by synthesizing onto supports oligomers of a predetermined length (e.g. length (6), i.e. forming 6mers). Each position in each 6mer is represented by one or the other of the binary groupings, overall forming a first sublibrary of synthesized oligomers. The sets of monomer groupings are then divided in a different manner into a second pair of binary groupings, and again oligomer mixtures are formed based on this second pair, thereby generating a second sublibrary of synthesized oligomers. A third sublibrary is similarly generated, using a third pair of binary groupings. The three pairs of binary groupings are defined such that any two monomer groupings are present together in only one binary grouping of one sublibrary. The sublibraries are reacted in the known activity, and the highest contributors to the activity are determined. The oligomer mixtures apparently contributing most highly are cross-correlated to generate likely oligomers responsible for the high activity. These oligomers are then synthesized and tested for activity, either directly or by a reiteration of the method of the invention, thereby determining oligomers likely contributing to the activity with relatively few oligomers required to be synthesized.

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METHOD AND APPARATUS FOR EFFICIENT MIMOTOPE DISCOVERY

This invention relates to an efficient method for mimotope discovery, and in particular to a method for elucidating which of a large number of possible oligomers is responsible for a given, known activity. Mimotopes, *i.e.* oligomers that give rise to essentially the same activity as the naturally occurring ligand, can then be identified and synthesized.

Background of the Invention

In attempting to identify the oligomers (*e.g.* peptides) responsible for a given activity, screening procedures are used, often involving the synthesis of many different oligomers of a given length, and conducting assays to determine which of the oligomers is likely to contribute to the observed activity. The activity may be, for instance, binding activity with a particular receptor or enzyme.

Mimotope discovery refers to the identification of a novel oligomer (for example) that binds to a receptor. The oligomer may or may not resemble the natural ligand. Such an oligomer can be referred to as a mimotope (although if it were very similar to the native binding species it would be called an analog).

For instance, a peptide may be identified which binds strongly to a receptor which normally binds a totally unrelated compound. Such a peptide would be a mimotope with respect to the peptide that *normally* binds to that receptor. An example of this would be a peptide that binds to a receptor that recognizes adrenalin (epinephrine), which is an aromatic amine. Such a peptide would be an adrenalin mimotope. An example in nature is that of morphine (an alkaloid) and the enkephalins (a class of peptides) which both bind to the mu opioid receptor.

In some approaches, mimotope discovery involves the preparation of numerous oligomer mixtures, and each mixture is tested against the receptor preparation of interest. Through careful definition of the mixtures and correlation of results, oligomers that are likely to give rise to the observed activity may be elucidated.

For the screening of oligomer mixtures, it is useful (1) to minimize the number of times a given oligomer is represented in the total set so that individual mixtures contain the minimum number of oligomers possible, and (2) to group the oligomers such that the smallest number of mixtures gives high activity results. However, the number of test mixtures must also be limited so that valuable receptors are used efficiently.

If a study were performed with discrete peptides, each containing N residues and being prepared from n' (n-prime) monomers, then a total of $X' = n'^N$ (*i.e.*, n' or n-prime raised to the power N) peptides would need to be prepared. For example, if all possible 6mers were synthesized using 17 monomers, then the number of peptides required to be prepared would be $X' = 17^6 = 24,137,569$.

One way to reduce the number of syntheses and tests required is to perform the synthesis with monomer groupings, where (similar) monomers were grouped together into n mono groupings. The number of peptide mixtures that could be prepared is then given by $X = n^N$. For example, for 6mers, if all possible mixtures were prepared from 6 monomer groupings, then $X = 6^6 = 46,656$.

This number is still too large for synthesis and screening to be practical. However, the number of discrete peptide mixture to be synthesized could be reduced by grouping the monomer groupings into even larger groupings. For instance, the monomer groupings might be combined into larger grouping, such as by either: (1) using small groupings (or individual monomers) together with complete mixtures; or (2) by using a smaller selection of larger groupings. The former was performed in the original approach described by H. Mario Geysen in patent application PCT/AU85/00165, WO86/00991 on "Method of determining mimotopes"; see also H.M. Geysen et al., *A priori delineation of a peptide which mimics a discontinuous antigenic determinant*, Molecular Immunology, 23, pp. 709-715 (1986). Geysen's approach has been widely popularized by Richard Houghten; see R. A. Houghten et al., *Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery*, Nature, 354, pp. 84-86 (1991). (The foregoing application and articles, and the other articles, patents, and other references cited in this application, are incorporated herein by reference.) In either case, a great deal of the resolving power of the peptide library is lost.

Therefore, a strategy is needed which reduces the number of required syntheses and assay steps without sacrificing the resolution of the experiment, i.e. the degree to which a given peptide may be determined as being responsible for the known activity.

Summary of the Invention

The method of the present invention involves first selecting a set of monomers, which will be used to construct a set of oligomers which will be tested for a known activity, such as binding with a receptor. The oligomers may or may not bear resemblance to the native ligands, *i.e.* the compounds that naturally bind to the receptor. Accordingly, the monomers may or may not bear resemblance to chemical structure(s) present within the native ligand(s); indeed, these structures may not even be known.

The method of the invention can thus be carried out even if nothing is known about the native ligand, using oligomers that are not related in any way to the native ligand.

A set of such monomers is selected, and they are grouped, preferably according to similarity of chemical characteristics. These monomer mixtures are used to build binary groupings of the sets of monomer mixtures; that is, the set of monomer groupings are divided into two (hence binary) mutually exclusive groupings, each including some of the monomer groupings and not others. These binary groupings are used to form oligomers in numerous combinations on solid supports (for example, pins), with monomers in one of the two binary groupings present at each position in the oligomers. For instance, in an oligomer of length 6, if the first binary grouping is represented as 0 and the second is represented as 1, then all combinations of oligomers from 0-0-0-0-0-0 to 1-1-1-1-1-1 are formed, where for each 0 or 1 all the monomers in that group are represented in the final set of oligomers.

This process is repeated for a second, different binary division of the monomer groupings, and again for a third. The result is three sublibraries of oligomers of varying combinations of the selected monomers, synthesized on solid supports. Additional monomer groupings and/or additional sublibraries can be prepared as required.

Alternatively, the monomer groupings can be applied to resins, and the elucidation of the mimotopes can be carried out by the "split resin" approach, wherein the resins that

have been used in the preparation of the libraries are divided into discrete batches. These batches are reacted with individual monomers, and then remixed. Testing of the oligomers in this approach is done on the solution phase of the oligomers. This approach yields results as though the monomers were mixed, but avoids the problem of differential rates of coupling, hence variation in degree of monomer incorporation. This is a method that can be used with beaded resins and other beaded supports, but not with the pins used in the preferred embodiment of the invention described below. Aspects of the split resin method are described in Huebner *et al.*, U.S. Patent No. 5,182,366, incorporated herein by reference.

These oligomers (in either approach) are all reacted in the known activity, *e.g.* reacted with a receptor, and the resulting level of activity is determined (such as by an ELISA analysis). When the oligomers have been formed on solid supports, they may be retained on the supports for testing, or alternatively they may be cleaved from the supports and reconstituted into solutions of the respective oligomer mixtures for testing.

The amount of activity that each oligomer mixture of each sublibrary shows is used in a deconvolution procedure to determine which specific monomers at each of the six (*e.g.*) positions are likely to be responsible for the high level of activity.

These results drastically narrow down the breadth of search necessary to elucidate the responsible oligomer(s), and to synthesize mimotopes for further testing. The resolution obtainable is extremely high relative to the number of oligomers necessary to be synthesized, as applicant's testing (discussed below) has demonstrated.

Brief Description of the Drawings

Figure 1 represents the size of a library of peptide mixtures needed to isolate an active oligomer of size N (*i.e.* of length N in monomer units).

Figure 2 represents the reduction in library size as the number of components in each library mixture is increased.

Figure 3 represents the effective library size using sublibraries of mixtures according to the present invention.

Figure 4 represents an intersection of the sublibraries of Figure 3.

Figure 5 depicts an array of pins and wells used in the process for elucidating oligomers.

Figure 6 illustrates the exposure of the pins of Figure 4 to a receptor preparation.

Figures 7-9 reflect test results from application of the method of the invention to a known serum identified herein as S1479-1.

Figures 10-12 are graphs representing the degree of binding of sublibraries of peptide mixtures prepared according to the invention with serum S1479-1.

Figure 13 is a graph illustrating the degree to which particular points (residues) in high-binding peptides identified from Figures 6-8 are conserved.

Figure 14 illustrates a replacement net study conducted by applicant on the epitope S1479-1 for each of its six residues.

Description of the Preferred Embodiments

A simple approach to mimotope discovery would be to synthesize each of the oligomers (*e.g.* peptides) suspected of activity, and to screen all of the synthesized oligomers. As discussed above, this would require the synthesis of far too many oligomers to be practical.

Figure 1 represents symbolically the size of an oligomer library 10 containing a number X of such oligomer mixtures, where $X = n^N$, with n being the number of monomer groupings used in

the library construction and N being the length of the oligomer. Thus, this represents the size of a library needed to screen all possible variations of oligomers each having N residues, where

each residue has n possibilities. Such an oligomer might be represented as R_1-R_2-R_3-R_4-R_5-R_6, where each R_N may be any one of a number of different monomer groupings.

Another approach to elucidating a mimotope such as an oligomer (*i.e.* isolating it as being responsible for the known activity) would be to increase the number of components (oligomers) in each mixture in the library, and decrease the number of mixtures. This then decreases the total number of mixtures to be made and maintained in the library, as illustrated by the reduced-size libraries 20 and 30 in Figure 2, but decreases the potential

resolution of the system, since if a given mixture is active it is not possible to tell which of the many components of the mixture is responsible for the activity, without a number of deconvolution steps using progressively smaller mixtures. The larger the initial active mixture, the more deconvolution steps that may be required.

The present invention, by contrast, maintains the full resolution for n^N oligomers, while reducing the number of mixtures that have to be prepared and tested. Figure 3 represents a library of mixtures according to the invention, and is constructed in a manner described below.

The term "monomer" as used herein refers to a chemical entity which may be covalently linked to one or more other entities to form an oligomer. Exemplary monomers include D- and L-amino acids, saccharides, nucleotides, peptoids, and the like. In general, a suitable set of monomers will have first and second ends (*e.g.*, C-terminal and N-terminal ends, or 5' and 3' ends) suitable for attaching other monomers by means of standard chemical reactions (for example, condensation, nucleophilic displacement of a leaving group, and the like), and a diverse element (*e.g.*, amino acid side chain, nucleotide base, *etc.*). Terminal monomers need only one attachment site. Monomers may be formed "*in situ*", by the combination of two or more "submonomers" as described in WO94/06451, incorporated herein by reference.

The term "oligomer" refers to a compound which is generated from two or more monomers in combination. In the practice of the instant invention, oligomers will generally comprise about 2-50 monomers, preferably about 2-20, more preferably about 3-10 monomers. Oligomers may be linear, branched, or cyclic, and need not retain the original monomer structure apart from the diverse element.

Group theory basis for the method of the invention

Library Compression

The library 10 shown in Figure 1 (with $X = n^N$ mixtures) can be compressed without significant loss in resolution, by constructing a set of related sublibraries (A, B, C, ...). Each sublibrary encompasses all of the oligomer mixtures contained in library 10, but with differing distributions within each sublibrary. Following are the four types of mixtures or groupings utilized in the preferred embodiment of the invention:

(1) *Monomer groupings.* In the preferred method of the present invention, monomer groupings are composed of appropriately derivatized (*i.e.* protected) monomers; they may, as noted above, alternatively be formed using the split resin approach. They are incorporated into oligomers as described below, and are thereby chemically altered, but may in context continue to be referred to as "monomer groupings" for the sake of the group analysis used for the described method of oligomer elucidation. (For example, amino acid mixtures are incorporated into peptides, and are then referred to as amino acid residues.)

(2) *Binary groupings.* Binary groupings are groups created, in the preferred embodiment, by mixing monomer groupings together in order to synthesize a sublibrary. For each binary grouping, there is another binary grouping which is its counterpart. Again, they may be formed alternatively by using the split resin approach.

(3) *Oligomer mixtures.* Each mixture generated in the library is called an oligomer mixture herein. Each binary library, or "sublibrary", is composed of 2^N oligomer mixtures, which are used in the screening procedure.

(4) *Intersection mixtures.* An intersection mixture is a mixture of oligomers common to a set of oligomer mixtures, where

one oligomer mixture is drawn from each of the sublibraries; that is, an intersection mixture is the common intersection (in the sense of set intersection) of a number of oligomer mixtures. Intersection mixtures are identified in the screening process by inter-sublibrary comparison.

Compression is achieved by combining the monomer groupings into two larger binary groupings, with each pair of the larger groups being mutually exclusive. Furthermore, each sublibrary has a unique pair of binary groupings. Discrete mixtures are synthesized using every possible permutation of the two groups of monomer groupings. Consequently, each sublibrary contains 2^N discrete oligomer mixtures.

The binary groupings (of monomer groupings) are constructed by bisecting the total monomer set, as discussed in detail below. Prior to bisection, the monomer groupings are organized by a group transformation (symmetry operation) designed to systematically swap monomer groupings between the two (binary) groups. Each transformation is unique to each sublibrary. Although the reorganization and bisection can be performed by two matrix operators, the total monomer grouping set can be divided into two equal, mutually exclusive groups in a variety of ways.

If a single sublibrary were used in isolation, it would merely be a low-resolution library derived from two (large) monomer groupings, *i.e.* two large binary groupings. Resolution is recovered by making comparisons between individual mixtures within a plurality of sublibraries. This is best illustrated diagrammatically. Area W in Figure 4 represents all n^N oligomer mixtures (defined by monomer groupings a-f) which are common to sublibraries A, B and C. Active oligomer intersection mixture "m" is present in all sublibraries A, B and C as a component of the nonidentical discrete mixtures A', B' and C'. By comparing active mixtures A', B' and C', the common mixture (*i.e.* intersection mixture) "m" is elucidated, hence library resolution is restored by using the three dependent sublibraries. This example is very simplistic in that only a single mixture is assumed. In practice, this would be demonstrated using a number of highest-activity oligomer mixtures drawn from each sublibrary.

Sublibrary Requirements

For the comparative strategy to work, the number of comparisons of individual mixtures between sublibraries must equal or exceed the maximum number of monomer grouping assemblies possible. Hence, the minimum number of sublibraries required in a transformed group library, x_{min} is given by:

$2^{NXmin} \geq n^N$, so $X_{min} \geq \ln(n)/\ln(2)$

where x is an integer and n is an even integer.

Note that the number of discrete mixtures synthesized is still relatively small compared to the number within library 10, namely; the number of discrete mixtures in library 10 is $X_{min} \cdot 2^N$.

Sufficient sublibraries must exist so that no monomer grouping is paired with another given monomer grouping in all sublibraries, which enables each monomer grouping within an oligomer mixture to be identified by the comparative approach of the invention.

A desirable but nonessential requirement is that each monomer grouping be paired with every other monomer grouping at least once. This then enables mutually replaceable monomer grouping pairs to be easily identified. This is achieved by constructing additional sublibraries to satisfy this condition. Table 1 shows the sizes of some transformed group libraries:

Table 1: Sizes of Transformed Group Libraries

N	n	n^N	2^{NXmin}	X_{min}	2^N	$X_{min} \cdot 2^N$
3	4	64	64	2	8	16
	6	216	512	3	8	24
	8	512	512	3	8	24
	10	1000	4096	4	8	32
4	4	256	256	2	16	32
	6	1296	4096	3	16	48
	8	4096	4096	3	16	48
	10	10,000	65,536	4	16	64
5	4	1024	1024	2	32	64
	6	7776	32,768	3	32	96
	8	32,768	32,768	3	32	96
	10	100,000	1,048,576	4	32	128
6	4	4096	4096	2	64	128
	6	46,656	262,144	3	64	192
	8	262,144	262,144	3	64	192
	10	1,000,000	16,777,216	4	64	256

In this table:

$X_{min} = 2$ when $n = 4$;
$X_{min} = 3$ when $n = 6$ or 8; and
$X_{min} = 4$ when $n = 10$.

Note also that:

N = length of oligomer in monomer units;

n = number of monomer groupings used in oligomer synthesis;

$X = n^N$ (*i.e.* n raised to the power N), representing the maximum number of discrete mixtures that could be prepared, which is the same as the resolving power of the transformed group library; and

$X_{min} \cdot 2^N$ represents the total number of discrete mixtures that need to be synthesized for the specified parameters.

With n=6 and N=6, it can be seen that with the method of the present invention only 192 discrete mixtures must be made, while for a direct approach with comparable resolution 46,656 discrete mixtures would have to be made.

The value 2^{NXmin} represents the number of potential intersections between the sublibraries represented by the $(X_{min} \cdot 2^N)$ discrete mixtures. The 192 mixtures mentioned above have 262,144 intersections, which encompass the 46,656 intersections representing the oligomer mixtures that would have to be made under the aforementioned direct approach. (Note that in this case, 215,488 intersections are spurious, because for the present example only six, not eight, monomer groupings were used; *i.e.* only six of eight binary numbers, 000-111, were used to define monomer groupings.)

The maximum number of sublibraries that can be constructed from n monomer groupings is given by:

$$X_{max} = 0.5 \times C(n,n/2) = 0.5 \times n!/(n/2)!^2.$$

Since this number increases very quickly with increasing n, it would be impracticable to prepare all possible sublibraries; however, this is unnecessary, because many of the sublibraries would be degenerate.

In mimotope discovery, the activity of each oligomer mixture within each sublibrary is determined. The intersection mixtures that are identified using the high-activity oligomer mixtures encompass a set of compounds likely to contain high-activity oligomers. Potential constituent monomers and residue positions are identified in this process.

Such an intersection is illustrated in Figure 3, where library 40 has been broken down into sublibraries A and B (for the sake of example); the intersection 50 between these two sublibraries represents the set of mixtures that produced the expected activity, and thus should include the oligomer responsible for the activity.

Construction of sublibraries

It is presumed for this example that n=6 and N=6 as defined above, *i.e.* that six monomer groupings will be used to prepare a set of 6mers (oligomers of length 6). Instead of requiring the synthesis of 46,656 oligomer mixtures, the monomer groupings - which may be labeled a, b, c, d, e and f - are combined into two binary groups (group A_0 and group A_1) of three monomer groupings each, reducing the number of required syntheses to 2^6 (*i.e.* 2 to the 6th power) = 64. Group A_0 may be defined as including monomer groupings a, b and c, and group A_1 as including monomer groupings d, e and f.

Binary groupings A_0 and A_1 are used to construct sublibrary A; that is, sublibrary A is composed of the oligomers that are generated from these binary groupings. An oligomer mixture represented by the concatenation

$$A_\#A_\#A_\#A_\#A_\#A_\#$$

can be synthesized, where the # symbol represents either a 0 or a 1 in each position. Wherever A_0 appears, the binary grouping (a,b,c) is present, and wherever A_1 appears, the binary grouping (d,e,f) is present. For instance, a concatenation of the form

$$A_0A_1A_1A_1A_0A_1$$

would refer to an oligomer mixture of the form

(abc)-(def)-(def)-(def)-(abc)-(def).

Each of the monomer groupings (a, b, c, d, e, f) stands for one or more monomers, *e.g.* "a" may stand for the amino acids (V, I and L), "b" may stand for (Y, F and homoPhe), and so on. Table 5 below identifies the amino acids that were used for each of the monomer groupings (a-f) in experiments carried out by applicant.

Since $A_\#$ can stand for either one of two binary groupings, the set of all possible concatenations of $A_\#A_\#A_\#A_\#A_\#A_\#$, represents $2^6 = 64$ possible oligomer mixtures. That is, $A_\#A_\#A_\#A_\#A_\#A_\#$ refers to a set of 64 different oligomer mixtures, and may be represented binarily as the series (000000, 000001, 000010, ..., 111111), where a "0" refers to A_0 and a "1" refers to A_1. Accordingly, the binary grouping (abc) is present wherever a "0" appears, and the binary grouping (def) is present wherever a "1" appears.

Oligomer mixtures are prepared in a manner to be described below. The resulting mixtures are 6mers (for this embodiment), with a binary grouping A_0 or A_1 represented at each position in the 6mer. Since each binary grouping includes three monomer groups, and each monomer group in this embodiment includes up to three monomers, the number of 6mers actually formed includes all possible variations on each monomer at each position. (Of course, more than three monomer groups could have been used.) For instance, the oligomer mixture 011101 (for sublibrary A) represents a mixture including all possible variations on the 6mer (abc)-(def)-(def)-(def)-(abc)-(def), where each of the monomer group designations (a-f) represents all of the up to three monomers represented thereby.

As shown in calculations below, a single oligomer mixture such as $A_0A_1A_1A_1A_0A_1$ represents many thousands of actually synthesized 6mers.

The six monomer groups in the set (a,b,c,d,e,f) are arranged so that they can be divided into paired binary groupings (such as abc and def discussed above, or alternatively such as abd and cef, *etc.*) by a symmetry operation. As the organization of the monomer groupings into any binary grouping is usually not crucial in practice, there are 10 possible sets of binary groupings possible (20 possible groups, 10 possible pairs), since the combination of 6 items taken 3 at a time is 20: that is,

$$C(6,3) = 6!/3!x3! = 20.$$

The organization of the monomer groupings may become important in special cases. For instance, if monomer groups a and d might effectively replace one another, this can be brought out by pairing them together.

The present embodiment, for the sake of illustration, uses only three of these possible pairings of binary groupings, which are used to generate sublibraries A, B and C, and which can be derived from one another by a simple set of transformations. The transformation results in a pair of monomer groupings being swapped between groups 0 and 1. Sublibrary A is a pair of binary groupings (abc) and (def) as discussed above, and sublibraries B and C are defined in Table 2.

Table 2: Sublibrary Binary Group Composition

Sublibrary	Binary Grouping 0	Binary Grouping 1
A	(a, b, c)	(d, e, f)
B	(a, b, f)	(c, d, e)
C	(a, e, f)	(b, c, d)

In this notation, binary grouping B_0 includes the monomer groupings (a, b, f), binary grouping B_1 includes monomer groupings (c, d, e), and so on.

Each sublibrary is constructed using the two binary groupings specific to that sublibrary. Thus, binary groupings A_0 and A_1 are used to construct sublibrary A, binary groupings B_0 and B_1 are used to construct sublibrary B, and binary groupings C_0 and C_1 are used to construct sublibrary C (and so on, if there are additional sublibraries). Each

sublibrary thus has the same form as the others, and this form is expressible as a sequence of binary numbers, where each digit of each binary number represents the binary grouping incorporated into that position within a given oligomer mixture. Each binary number (*i.e.* string of binary digits) represents a discrete mixture within a given sublibrary.

For example, for sublibrary C, the designation 010100 represents a 6mer (oligomer of length 6), where monomers comprising the binary grouping C_0 (= a, e, f) have been coupled (in a manner to be described below) at positions 1, 3, 5 and 6, and monomers comprising binary grouping C_1 (= b, c, d) have been coupled at positions 2 and 4.

It will be appreciated that the composition of mixture 010100 in a given sublibrary will depend upon the particular monomer groupings used in its construction. Each sublibrary is represented by the same set of binary numbers (000000 through 111111), but a given binary number represents a different discrete oligomer mixture, depending on which sublibrary it is being used in reference to.

If a set of oligomer mixtures is prepared from each of the above mixture sets A, B and C, a total of 3 x 64 = 192 oligomer mixtures (which may be designated A', B' and C') are obtained. These may all be tested for the desired activity, and different mixtures (from the set 000000 through 111111) in each sublibrary will be found to lead to differing levels of activity, for example different binding levels with a given antibody or other receptor. The manner in which these mixtures are identified is discussed below.

If, for example, a high-activity mixture is found the mixture 001001 of sublibrary A was that represented by 001001, the mixture 101101 of sublibrary B, and the mixture 110110 of sublibrary C, then examination of Table 2 reveals that the mixture likely to be responsible for the activity is "cbecbe". This is determined by reading the first digit of each of the binary numbers of A, B and C, then the second, and so on:

A: 001001
B: 101101
C: 110110

In this case, this inspection yields the following binary numbers in sequence: 011-001-110-011-001-110. The first monomer grouping in the likely oligomer sequence, then, is represented by the motif 001 (or A_0-B_1-C_1), and the only monomer grouping common to

those three mixtures is, in fact "c". The second monomer grouping is in like manner identified as monomer grouping "b", and so on.

Each monomer grouping within the binary groups 0 and 1 (for each sublibrary used in the sublibrary construction) can similarly be identified by a unique motif. Reorganization of Table 2 yields these motifs. A table of such motifs can be generated, with a unique binary number for each monomer grouping:

Table 3. Monomer groupings and corresponding binary number motifs*

*(for n=6, X_{min} = 3)

Monomer grouping	Sublibrary: A	B	C
a	0	0	0
b	0	0	1
c	0	1	1
d	1	1	1
e	1	1	0
f	1	0	0

Other motifs, where two or more monomer groupings can be tolerated at a given position, can be deduced from Table 3.

For this case, where n = 6, there are no monomer groupings corresponding to the combinations 010 and 101; this is because $2^{NXmin} > n^N$. If eight monomer groupings had been defined in the library construction, all motifs would define unique monomer groupings, since in this case $2^{NXmin} = n^N$. Ten or twelve monomer groupings would require an additional sublibrary (D) for deconvolution.

In general, to cover more cases additional mixtures would be needed. A more extensive test may be designed, *e.g.*, by increasing the number ("n") of monomer groupings, or by increasing the number of monomers within each monomer grouping.

Thus, in cases where two particular monomer groupings are never grouped together, or never grouped separately, a recognizable motif is not generated using these three sets (*e.g.* a/d, b/e and c/f). This situation can be rectified by generating two more pairs of binary groups to give a total of 5 x 64 = 320 oligomer mixtures (note that in the above example only 3 of 10 possible binary group pairs have been constructed).

Motifs for all possible paired monomer groupings are given in Table 4. If three or more monomer groupings need to be identified, then additional pairs of groups are required; however, these positions would be highly replaceable and of less interest than the highly conserved positions. It should be noted that motifs would be defined by the high-affinity oligomer mixtures from each set.

Table 4. Motifs for combinations of monomer groupings.

Monomer grouping	Sublibrary A	B	C		D	E
a	0	0	0		0	0
b	0	0	1		0	1
c	0	1	1		1	1
d	1	1	1		1	0
e	1	1	0		0	0
f	1	0	0		1	1
a/b	0	0	# (0 or 1)		0	#
a/c	0	#	#		#	#
a/d	#	#	#		#	0
a/e	#	#	0		0	0
a/f	#	0	0		#	#
b/c	0	#	1		#	1
b/d	#	#	1		#	#
b/e	#	#	#		0	#
b/f	#	0	#		#	1
c/d	#	1	1		1	#
c/e	#	1	#		#	#
c/f	#	#	#		1	1
d/e	1	1	#		#	0
d/f	1	#	#		1	#
e/f	1	#	0		#	#

Sublibrary D: 0 = [e, a, b]; 1 = [c, d, f]

Sublibrary E: 0 = [a, d, e]; 1 = [b, c, f]

Here, sublibraries D and E are exemplary; other sublibraries could have been used.

It is also possible to carry this out with four monomer groupings, in which case three sets of paired groups will provide all of the information available from four monomer groupings in 192 oligomer mixtures (though the resolution would be lower than for an n = 6 library). If these were prepared by combination of single monomer groupings as in prior approaches, 4096 oligomer mixtures would be required.

Dominant residues

If certain "dominant" residues are critical for oligomer activity, it could then be expected that their positions within the active oligomer mixtures to be intolerant of change, *i.e.* if other oligomers are substituted at those positions, the activity would diminish significantly. Therefore, if the oligomer mixtures for each sublibrary are ordered by degree of activity demonstrated in a binding experiment, the binary patterns seen for the top binders would be highly conserved at those positions.

For example, if "a" is an absolute requirement at a given position (such as the third position) for binding activity of a given oligomer to take place, one would expect all binding oligomer mixtures to have the general form [## 0 ###], where the symbol # indicates that the numeral may be either a 0 or a 1.

In this example, since the motif for a is 000 (see Table 3), one would expect to observe the following patterns with binding oligomer mixtures obtained from the three sublibraries:

A': ## 0 ###

B': ## 0 ###

C': ## 0 ###

where # represents either 0 or 1. As indicated above, the motif "000" is indicative of "a" at the third oligomer position; that is, if each of the active peptide mixtures identified in sublibraries A', B' and C' has its respective binary grouping represented at position 3, this means that position 3 for the A' set of mixtures included (a, b, c); for the B' set of mixtures it included (f, a, b); and for the C' set of mixtures it included (e, f, a). Since the only common monomer grouping among these three sets is monomer grouping "a", it may be concluded that the active oligomer(s) contain, at position 3, one or more monomers from monomer grouping "a".

If in addition "e" were also an absolute requirement (for binding activity) at, for instance, position 5, up to 16 active oligomer mixtures may be obtained in each series A', B' and C', of the form:

A': ## 0 # 1 #
B': ## 0 # 1 #
C': ## 0 # 0 #

where # represents 0 or 1. That "e" is present at position 5 is clear because the A'-B'-C' motif at that position is 110, and Table 3 shows that the only common monomer grouping for these three groups (A_1, B_1, C_0) is "e".

In this case, since "a" is conserved at the third position and e at the fifth position, one would expect the complements of each of the above binary numerals to lead to very low activity: *e.g.* A'= ##1#0#, B'=##1#0# and C'=##1#1# should all yield low binding results. This is in fact the case, as determined by applicant's experiments, discussed below. (This argument assumes that the binding core of residues remains fixed relative to the left and right termini of the test oligomer mixtures. In the case of peptides, these are the N- and C-termini, respectively.)

If the residues that are responsible for activity can be encompassed by an oligomer containing fewer than N residues, then residues at either or both of the N-and C-termini may play little role in the binding activity. In such cases, a "frame-shifting" of the residues may be possible. For instance, if a binding residue pattern of #0011# is observed (assuming for the moment that the 0011 sequence is responsible for the binding), the residue pattern 0011## may bind equally or nearly as well, if the sequence of oligomers primarily responsible for the binding activity is the group 0011. This example might yield good binding results for all of the following sequences:

001100	000110	000011
001101	00111	010011
001110	100110	100011
001111	100111	110011

In this case, there may be no strongly conserved *position*, but a conserved *pattern* of oligomers may be observed, once one takes into account the possibility of frame-shifting.

To identify a conserved position, it is instructive to compare patterns within a given sublibrary to determine the effect of substitution of a single binary grouping for the other binary grouping at that position. For example, if the activity of 001001 in library A (*i.e.* the mixture A_0-A_0-A_1-A_0-A_0-A_1) is compared with the activity of 101001 (*i.e.* the mixture A_1-A_0-A_1-A_0-A_0-A_1), then it can be determined how important the binary grouping A_0 is in the first position; if a similar level of activity is demonstrate by 101001, then it would appear that A_0's presence in the first position is not crucial to the binding activity (or else A_0 and A_1 both contain one or more monomers that are equally acceptable in that position within the oligomer(s) in question); whereas if the sequence 101001 shows significantly lower activity, then it would appear that A_0's presence in the first position is important to the binding activity.

A systematic comparison of each sequence can be carried out with other sequences in the same library, each having the complement of the one of the binary digits of the first sequence. Thus, the sequence 101001 would be compared with six other mixtures in the same sublibrary:

Complement Comparisons

101001
101000
101011
101101
100001
111001
001001

The results of such comparisons reflect the relative importance of each of the residues in the elucidated active mixtures. This is borne out by the experimental data discussed below.

Mimotope discovery

The above group theory and general approach described above with respect to oligomers in general can be used with peptides, or indeed any amino acid or other monomer desired, such as groupings of amines, carboxylic acids, sugars, nucleic acids, and in general any chemical building blocks that may be linked in oligomers. See for example, WO91/19735, incorporated herein by reference, for a description of non-peptide oligomers ("peptoids") within the scope of this invention. Oligomers in the range of 3mer to >6mers may easily be studied by this approach, with the number of primary monomer groupings ranging from four and up (where n should be an even integer).

The method of the invention may be used in either support-bound or solution-phase assays, *i.e.* oligomers retained on solid supports or cleaved therefrom for testing. In general, the sublibraries are prepared, and are assayed by contacting the oligomers with a target (*e.g.*, a receptor, enzyme, oligonucleotide, whole cell, organism, tissue culture, and the like) and detecting the presence or absence of a desired interaction between the target and the oligomer. The desired activity may be inhibition or potentiation of an enzymatic activity (for example, inhibition of dihydrofolate reductase activity), growth or survival of a

cell line, binding to a specific protein (for example, binding to antisera characteristic of a particular infection, used to diagnose presence of the infection in the serum donor), and the like. In its simplest form, the oligomers are presented bound to a solid phase and are contacted with the target. The target is allowed to bind, and non-binding target is washed away. The presence of target bound to specific oligomers is detected, for example, by using an anti-target antibody labeled with a detectable label (*e.g.*, radioactive atoms or enzymes capable of catalyzing a detectable color change).

Alternative embodiments of the method include: (1) application to oligomer mixtures where one or more positions are invariant; and (2) use in combination with complete mixtures, *i.e.* a hybrid between the defined-position strategy and the transformed-group strategy.

Applicant has conducted initial experiments using relatively simple monomer groupings, including L-amino acids and a well-understood serum (containing a monoclonal antibody) requiring a well-characterized epitope (Serum 1479-1, having epitope = DVPDYA). Upon validation, D/L mixtures and additional side chain diversity should be considered. Following is a description of the initial experiment carried out by applicant. In the experimental procedures described below, the oligomers in question are peptides, and the monomers are amino acids.

Experimental Procedure and Results

In an experiment to apply the method of the invention, applicant synthesized peptide mixtures by preparing six equimolar solutions of N-alpha 9-fluorenylmethoxycarbonyl (Fmoc) protected L-amino acids (the monomer groupings). Definitions and side-chain protection are listed below.

1-letter Code	3-letter Code	Standard Name	Derivative Used in Peptide Synthesis in This Study
A	Ala	Alanine	Fmoc-L-Ala-OH
C	Cys	Cysteine	(not used in this study)
D	Asp	Aspartic acid	Fmoc-L-Asp(OtBu)-OH
E	Glu	Glutamic acid	Fmoc-L-Glu(OtBu)-OH
F	Phe	Phenylalanine	Fmoc-L-Phe-OH
G	Gly	Glycine	Fmoc-Gly-OH
H	His	Histidine	Fmoc-L-His(Boc)-OH
hF	homoPhe	Homophenylalanine	Fmoc-L-homoPhe-OH
I	Ile	Isoleucine	Fmoc-L-Ile-OH
K	Lys	Lysine	Fmoc-L-Lys(Boc)-OH
M	Met	Methionine	(not used in this study)
N	Asn	Asparagine	Fmoc-L-Asn(Trt)-OH
P	Pro	Proline	Fmoc-L-Pro-OH
Q	Gln	Glutamine	Fmoc-L-Gln(Trt)-OH
R	Arg	Arginine	Fmoc-L-Arg(Pmc)-OH
S	Ser	Serine	Fmoc-L-Ser(tBu)-OH
T	Thr	Threonine	Fmoc-L-Thr(tBu)-OH
V	Val	Valine	Fmoc-L-Val-OH
W	Trp	Tryptophan	(not used in this study)
Y	Tyr	Tyrosine	Fmoc-L-Tyr(tBu)-OH

Cys, Met and Trp, which might be present in a more comprehensive test, were excluded to avoid oxidation problems peculiar to these residues. Homophenylalanine (homoPhe) was included in monomer grouping b as a substitute for Trp, and Thr was excluded from group d to keep its number of amino acids to three. In this experiment, only two monomers were used in group c. The experiment thus involved constructing the following six equimolar sets of L-amino acids:

Table 5: the mixture components

a = V, I, L	b = Y, F, homoPhe
c = D, E	d = S, N, Q
e = A, G, P	f = H, K, R.

Note that in this case the monomers were grouped as follows:

a = aliphatic amino acids;

b = aromatic amino acids;

c = acidic amino acids;

d = hydrophilic amino acids;

e = small amino acids; and

f = basic amino acids.

Preparation of pins.

Synthesis was performed on a pin grafted with methacrylic acid and dimethyl-acrylamide. The surface was derivatized for synthesis in a standard manner, as described in N.J. Maeji, R.M. Valerio, A.M. Bray, R. A. Campbell, H.M. Geysen (1994), *Grafted supports used with the multipin method of peptide synthesis*, Reactive Polymers, 22, 203-212. See also US 4,833,092. Prior to synthesis, the pins were derivatized with Boc-hexamethylene-1,6-diamine, Boc deprotected by treatment with trifluoroacetic acid, treated with triethylamine in dimethylformamide, washed with dimethylformamide and methanol and air dried, and coupled with Fmoc-Gly- OH/diisopropylcarbodiimide/1-hydroxybenzotriazole (60 mM, 60 mM, 72 mM) in DMF to give a Gly loading of 1 micromole/detachable pin head (see R.M. Valerio, A.M. Bray, R.A. Campbell, A. DiPasquale, C. Margellis, S.J. Rodda, H.M. Geysen and N.J. Maeji (1993), *Multipin peptide synthesis at the micromole scale using 2-hydroxyethyl methacrylate grafted polyethylene supports*, International Journal of Peptide and Protein Research, 42, 1-9.

Preparation of amino acid mixtures (monomer groupings a - f).

The mixtures were made substantially equimolar in the following manner, using standard amino acid stock solutions. The volume of each required is 1/6 (because there are six mixtures) x 6 (length of peptide) x 0.06 (ml/coupling) x 192 (number of peptides) = 11.52 ml. Allowing for transfers, *etc.*, the volume is thus at least about 13.5 ml. Stock solutions of the 17 Fmoc-protected amino acids in dimethylformamide (250 mM) were used to prepare the monomer grouping stock solutions a, b, c, d, e and f.

Where the solution contained three amino acids, 4.50 mL of each respective amino acid stock solution was used to prepare 13.5 mL of monomer grouping stock solution. In the case of solution "c", 6.75 mL each of Fmoc-L-Asp(OtBu)-OH and Fmoc-L-Glu(OtBu)-OH stock solutions were used to give 13.5 mL of stock solution.

The working stock solutions A_0', A_1', B_0', B_1', C_0' and C_1' were constructed as follows:

A'(0) {or, alternatively notated, A_0'} = a (4 ml) + b (4 ml) + a (4 ml)

A'(1) {or A_1'} = d (4 ml) + e (4 ml) + f (4 ml)

B'(0) {or B_0'} = a (4 ml) + b (4 ml) + f (4 ml)

B'(1) {or B_1'} = c (4 ml) + d (4 ml) + e (4 ml)

C'(0) {or C_0'} = a (4 ml) + e (4 ml) + f (4 ml)

C'(1) {or C_1'} = b (4 ml) + c (4 ml) + d (4 ml)

Synthesis of Peptide Mixtures.

The synthesis of peptide mixtures was then performed as follows:

Fmoc deprotection. The pins were treated with 20% piperidine/dimethylformamide (50 mL/96 pins) for 30 minutes. The pins were then washed with dimethylformamide (50 mL/96 pins) for 5 minutes. The pins were then fully immersed into methanol and then washed with two further lots of methanol (100 mL/96 pins) and then air dried.

Coupling amino acid mixtures. Amino acid stock solutions A_0', A_1', B_0', B_1', C_0' and C_1' were added to the wells of two 96 well polypropylene microtitre trays (60 microlitres/well) in the dispensing patterns presented in Table 6 below. A solution of BOP/hydroxybenzotriazole/N-methylmorpholine (166 mM/166 mM/250 mM) in dimethylformamide was then added to each well (90 microlitres/well). The pins were then immersed into the solution and allowed to react for at least 2 hours. The pins were then removed from the wells and washed with dimethylformamide (50 mL/96 pins) and methanol (2 x 50 mL/96 pins), and air dried.

Fmoc deprotection and coupling cycles were performed alternatively until 6mer mixtures as indicated in Table 7 were assembled. The pins were then Fmoc deprotected and acetylated with acetic anhydride/triethylamine/dimethylformamide (2:1:50, v/v/v) (50 mL/96 pins) for 45 minutes. The pins were then washed with methanol and air dried.

Table 6: Dispensing Patterns for Amino Acid Stock Solutions

Coupling #1

Tray 1	Tray 2
A0 A0 A0 A0 A0 A0 B0 B0	B0 B0 B0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 A1 B1 B1	B1 B1 B1 C1 C1 C1 C1 C1
A0 A0 A0 A0 A0 A0 B0 B0	B0 B0 B0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 A1 B1 B1	B1 B1 B1 C1 C1 C1 C1 C1
A0 A0 A0 A0 A0 B0 B0 B0	B0 B0 B0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 B1 B1 B1	B1 B1 B1 C1 C1 C1 C1 C1
A0 A0 A0 A0 A0 B0 B0 B0	B0 B0 B0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 B1 B1 B1	B1 B1 B1 C1 C1 C1 C1 C1
A0 A0 A0 A0 A0 B0 B0 B0	B0 B0 C0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 B1 B1 B1	B1 B1 C1 C1 C1 C1 C1 C1
A0 A0 A0 A0 A0 B0 B0 B0	B0 B0 C0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 B1 B1 B1	B1 B1 C1 C1 C1 C1 C1 C1

Coupling #2

Tray 1	Tray 2
A0 A0 A0 A0 A0 A0 B0 B0	B0 B0 B0 C0 C0 C0 C0 C0
A0 A0 A0 A0 A0 A0 B0 B0	B0 B0 B0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 A1 B1 B1	B1 B1 B1 C1 C1 C1 C1 C1
A1 A1 A1 A1 A1 A1 B1 B1	B1 B1 B1 C1 C1 C1 C1 C1
A0 A0 A0 A0 A0 B0 B0 B0	B0 B0 B0 C0 C0 C0 C0 C0
A0 A0 A0 A0 A0 B0 B0 B0	B0 B0 B0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 B1 B1 B1	B1 B1 B1 C1 C1 C1 C1 C1
A1 A1 A1 A1 A1 B1 B1 B1	B1 B1 B1 C1 C1 C1 C1 C1
A0 A0 A0 A0 A0 B0 B0 B0	B0 B0 C0 C0 C0 C0 C0 C0
A0 A0 A0 A0 A0 B0 B0 B0	B0 B0 C0 C0 C0 C0 C0 C0
A1 A1 A1 A1 A1 B1 B1 B1	B1 B1 C1 C1 C1 C1 C1 C1
A1 A1 A1 A1 A1 B1 B1 B1	B1 B1 C1 C1 C1 C1 C1 C1

Coupling #3

Tray 1	Tray 2
A0 A1 A0 A1 A0 A1 B0 B1	B0 B1 B0 C1 C0 C1 C0 C1
A0 A1 A0 A1 A0 A1 B0 B1	B0 B1 B0 C1 C0 C1 C0 C1
A0 A1 A0 A1 A0 A1 B0 B1	B0 B1 B0 C1 C0 C1 C0 C1
A0 A1 A0 A1 A0 A1 B0 B1	B0 B1 B0 C1 C0 C1 C0 C1
A1 A0 A1 A0 A1 B0 B1 B0	B1 B0 B1 C0 C1 C0 C1 C0
A1 A0 A1 A0 A1 B0 B1 B0	B1 B0 B1 C0 C1 C0 C1 C0
A1 A0 A1 A0 A1 B0 B1 B0	B1 B0 B1 C0 C1 C0 C1 C0
A1 A0 A1 A0 A1 B0 B1 B0	B1 B0 B1 C0 C1 C0 C1 C0
A0 A1 A0 A1 A0 B1 B0 B1	B0 B1 C0 C1 C0 C1 C0 C1
A0 A1 A0 A1 A0 B1 B0 B1	B0 B1 C0 C1 C0 C1 C0 C1
A0 A1 A0 A1 A0 B1 B0 B1	B0 B1 C0 C1 C0 C1 C0 C1
A0 A1 A0 A1 A0 B1 B0 B1	B0 B1 C0 C1 C0 C1 C0 C1

Coupling #4

Tray 1								Tray 2							
A0	A1	A1	A0	A0	A1	B1	B0	B0	B1	B1	C0	C0	C1	C1	C0
A0	A1	A1	A0	A0	A1	B1	B0	B0	B1	B1	C0	C0	C1	C1	C0
A0	A1	A1	A0	A0	A1	B1	B0	B0	B1	B1	C0	C0	C1	C1	C0
A0	A1	A1	A0	A0	A1	B1	B0	B0	B1	B1	C0	C0	C1	C1	C0
A0	A0	A1	A1	A0	B0	B1	B1	B0	B0	B1	C1	C0	C0	C1	C1
A0	A0	A1	A1	A0	B0	B1	B1	B0	B0	B1	C1	C0	C0	C1	C1
A0	A0	A1	A1	A0	B0	B1	B1	B0	B0	B1	C1	C0	C0	C1	C1
A0	A0	A1	A1	A0	B0	B1	B1	B0	B0	B1	C1	C0	C0	C1	C1
A1	A0	A0	A1	A1	B0	B0	B1	B1	B0	C0	C1	C1	C0	C0	C1
A1	A0	A0	A1	A1	B0	B0	B1	B1	B0	C0	C1	C1	C0	C0	C1
A1	A0	A0	A1	A1	B0	B0	B1	B1	B0	C0	C1	C1	C0	C0	C1
A1	A0	A0	A1	A1	B0	B0	B1	B1	B0	C0	C1	C1	C0	C0	C1

Coupling #5

Tray 1								Tray 2							
A0	A0	A1	A0	A1	A1	B0	B1	B0	B0	B1	C0	C1	C1	C0	C1
A0	A0	A1	A0	A1	A1	B0	B1	B0	B0	B1	C0	C1	C1	C0	C1
A0	A0	A1	A0	A1	A1	B0	B1	B0	B0	B1	C0	C1	C1	C0	C1
A0	A0	A1	A0	A1	A1	B0	B1	B0	B0	B1	C0	C1	C1	C0	C1
A0	A1	A1	A0	A1	B0	B0	B1	B0	B1	B1	C0	C1	C0	C0	C1
A0	A1	A1	A0	A1	B0	B0	B1	B0	B1	B1	C0	C1	C0	C0	C1
A0	A1	A1	A0	A1	B0	B0	B1	B0	B1	B1	C0	C1	C0	C0	C1
A0	A1	A1	A0	A1	B0	B0	B1	B0	B1	B1	C0	C1	C0	C0	C1
A0	A1	A0	A0	A1	B0	B1	B1	B0	B1	C0	C0	C1	C0	C1	C1
A0	A1	A0	A0	A1	B0	B1	B1	B0	B1	C0	C0	C1	C0	C1	C1
A0	A1	A0	A0	A1	B0	B1	B1	B0	B1	C0	C0	C1	C0	C1	C1
A0	A1	A0	A0	A1	B0	B1	B1	B0	B1	C0	C0	C1	C0	C1	C1

Coupling #6

Tray 1								Tray 2							
A0	A0	A0	A1	A1	A1	B0	B0	B1	B1	B1	C0	C0	C0	C1	C1
A0	A0	A0	A1	A1	A1	B0	B0	B1	B1	B1	C0	C0	C0	C1	C1
A0	A0	A0	A1	A1	A1	B0	B0	B1	B1	B1	C0	C0	C0	C1	C1
A0	A0	A0	A1	A1	A1	B0	B0	B1	B1	B1	C0	C0	C0	C1	C1
A0	A0	A0	A1	A1	B0	B0	B0	B1	B1	B1	C0	C0	C1	C1	C1
A0	A0	A0	A1	A1	B0	B0	B0	B1	B1	B1	C0	C0	C1	C1	C1
A0	A0	A0	A1	A1	B0	B0	B0	B1	B1	B1	C0	C0	C1	C1	C1
A0	A0	A0	A1	A1	B0	B0	B0	B1	B1	B1	C0	C0	C1	C1	C1
A0	A0	A1	A1	A1	B0	B0	B0	B1	B1	C0	C0	C0	C1	C1	C1
A0	A0	A1	A1	A1	B0	B0	B0	B1	B1	C0	C0	C0	C1	C1	C1
A0	A0	A1	A1	A1	B0	B0	B0	B1	B1	C0	C0	C0	C1	C1	C1
A0	A0	A1	A1	A1	B0	B0	B0	B1	B1	C0	C0	C0	C1	C1	C1

Here, A0 refers to sublibrary A_0, A1 refers to sublibrary A_1, *etc.* Each position in each coupling in Table 6 represents one well 40 as depicted in Figure 5.

The following peptide mixtures were prepared by successive coupling to the pins as noted above, using each set of mixtures A(0,1), B(0,1) and C(0,1):

Table 7

000000	000001	000010	000011
000100	000101	000110	000111
001000	001001	001010	001011
001100	001101	001110	001111
010000	010001	010010	010011
010100	010101	010110	010111
011000	011001	011010	011011
011100	011101	011110	011111
100000	100001	100010	100011
100100	100101	100110	100111
101000	101001	101010	101011
101100	101101	101110	101111
111000	111001	111010	111011
111100	111101	111110	111111

The above peptide mixtures may be interpreted as follows: mixture 000000 for the sublibrary A set is prepared from six successive couplings of the A_0 mixture to a given pin, which as noted above includes the monomer groupings (a, b, c) mixed together (and each of a, b and c includes the amino acids noted in Table 5 above).

Thus, a portion of A_0 is placed in designated wells 40 of a reaction tray 50 such as that shown in Figure 5 (and as represented by the top left well -- and indeed in all wells represented by "A0" -- in Coupling 1, Tray 1 in Table 6). The portion of A_0 is coupled to a pin 80 attached to a carrier 90 by exposing the pin to the portion (see Figure 5), *i.e.* by placing the pins into the solutions in the wells. In this way, A_n is coupled (covalently

bonded) to the pins (solid supports). At the same time, A_1, B_0 and B_1 are coupled to other pins (see Table 6, Coupling #1, Tray 1). In Coupling #2, the second set of sublibrary mixtures is used in the wells (see Table 6, Coupling #2, Tray 1). The oligomers are thereby synthesized on the pins.

Note that, while an eight-by-eight array of pins and wells is represented in Figure 5, any convenient configuration may be used. For instance, in the procedure actually carried out using the wells represented in Table 6, and eight-by-twelve array was used.

This may be represented as 0-[support], where [support] represents the solid support, and 0 represents the addition of A_0. An array of pins is used to build up the oligomers, in a conventional fashion. It will be appreciated that multiple peptide mixtures are built up simultaneously in this fashion, namely 64 at a time for each tray listed in Table 6. Thus, very rapidly thousands of oligomers are formed on the pins, and these oligomers are to be used in the testing.

Although pins are used in this experiment, other synthesis media suitable for solid-phase synthesis could equally well be used, such as "tea bags", *i.e.* mesh packets of resin. Alternative support structures suitable for use in the method of the invention include appropriately derivatized glass, functionalized cellulose paper, polymer-grafted plastics, and the like. If resin is used, then the split resin approach may be used, as mentioned above in the Summary of the Invention.

Each amino acid in the mixture such as A_0 will normally include a protecting group, as indicated above, which is removed in conventional fashion at this point. Then, additional A_0 is coupled (see the top left well of Tray 1 in Coupling 2 of Table 6, and all wells in Coupling 2 marked "A_0"), so that the oligomer attached to the solid support is 0-0-[support]. This is repeated, until an entire chain is built up of 0-0-0-0-0-0-[support], representing A_0-A_0-A_0-A_0-A_0-A_0 for the pin that is immersed into the top left well in Table 6, Tray 1.

Chemically, this concatenation leads to the formation of $8^6 = 262,144$ peptides. This is because there are eight amino acids at each of the six positions of the chain A_0-A_0-A_0-A_0-A_0-A_0, since A_0 represents the group (a,b,c), which together comprise the amino acids (a = V, I, L); (b = Y, F, homoPhe); and (c = D, E). Each of those amino acids

couples with the preceding residue. Thus, when A_0 is coupled at the second position, 64 dimers are formed. Coupling of the third residue then gives 512 trimers, and so on up to 262,144 oligomers by the time the final (sixth) coupling is performed.

Similarly large numbers of peptides are present in each of the mixtures represented by 000001 through 111111. The lowest number of peptides in any mixture will be 8^6=262,144 (for any mixture where every position includes the monomer grouping "c", which only has two amino acids in this example), and the highest number is 9^6=531,441 (for any mixture where all positions include only monomer groupings with three amino acids each). Other mixtures will contain intermediate numbers of peptides, such as, for A_1-A_1-A_0-A_1-A_0-A_0, a total of 9x9x8x9x8x8 = 373,248 peptides (since A_1 includes nine amino acids, and A_0 includes eight).

Other library sizes, such as 4mer and 5mer libraries, could also be constructed, using L- and D- amino acids, and other sizes of binary groupings -- such as four and six monomer groupings per binary grouping -- may be used. These shorter libraries will take up fewer pins than the experiment described above.

These peptide mixtures were prepared on a non-cleavable high-loading crown surface for conventional pin ELISA testing against serum S1479-1. "ELISA" stands for "enzyme-linked immunoabsorbant assay". For a discussion of the ELISA testing/epitope analysis in this setting, see H.M. Geysen et al., *Strategies for epitope analysis using peptide synthesis*, "Journal of Immunological Methods", 102, pp. 259-275.

The three sublibraries A, B and C, prepared as discussed above, each included 64 mixtures, with each mixture including between 262,144 and 531,441 peptides, as explained. Thus, the resultant library had the parameters n=6, N=6 and X_{min} = 3. (Other parameters could have been used, such as n=8, N=6 and x=4. The choice of x=4 allows for all monomer grouping pairings.)

"Serum 1479-1" is applicant's designation for an antihemagluttin monoclonal antibody (IgG) preparation available (as serum "17D09") from the Scripps Clinic and Research Foundation. Serum S1479-1 is well understood (see Figure 14), and its antibody epitope is known to be DVPDYA. See I.A. Wilson *et al.*, *The structure of an antigenic determinant in a protein*, "Cell", 37, pp. 767-778 (1984); and J.M. Rini et al., "Science",

255, pp. 959-965 (1992). Thus, it makes a good test subject to examine the efficacy of the method of the invention.

The ELISA test was carried out according to the method described in the aforementioned Geysen article (*"Strategies for epitope analysis using peptide synthesis"*). The three sublibraries were formed on 192 pins (64 per sublibrary), and the pins were exposed to serum S1479-1, as illustrated in Figure 6, wherein the pins 80 mounted on holder 90 are submerged into a container 60 partially filled with a liquid 70 containing the serum.

After exposure to the serum preparation and rinsing to remove unbound antibody, the block is similarly submerged in a solution containing a horseradish peroxidase-labelled anti-IgG detecting antibody. The detecting antibody binds to the S1479-1 monoclonal antibody. Hence, pins that contain peptides to which the monoclonal antibody binds also bind the detecting antibody.

Following washing, the enzyme is detected by immersing the pins into microtitre plate wells which contain an enzyme substrate solution.

Alternatively, the target oligomers may be cleaved from the solid phase synthesis support by applying a cleavage reagent (usually a volatile acid), which is then removed, generally by evaporation. The dried peptides are then redissolved in a solution appropriate for subsequent testing.

In the preferred embodiment, the enzyme substrate solution into which the pins are immersed may be ABTS (Boehringer Mannheim cat. no 122661, 0.5 g/L) and hydrogen peroxide (120 vol, 0.3 mL/L) in pH 4 phosphate/citrate buffer. Color development was stopped simultaneously in all wells by removing the pins from the substrate solution. Absorbances were measured on a Titertek Multiskan MC plate reader at 405 nm against a reference wavelength of 492 nm.

Each pin yields an absorbance value indicative of the relative binding strength of the peptide mixture on that pin with the antibody under study. Such results are shown in Figures 7-9. Figure 7 shows the results for sublibrary A; it will be noted that the second column includes an entry for each of the binary numbers listed in Table 7 above. The first column of Figure 7 lists the ELISA absorbance results (in milliabsorbance units). Thus,

mixture A_0-A_0-A_1-A_0-A_0-A_1 showed the highest binding with serum S1479-1, with an ELISA absorbance value of 611. The next highest binding occurred with A_0-A_0-A_0-A_0-A_0-A_1, and the least with A_1-A_0-A_1-A_1-A_1-A_1.

The other columns of Figure 7 show the results of calculating the complement comparisons (as described above), with each entry representing the ratio of (1) the ELISA value for the mixture listed at the left, with (2) the ELISA value of the mixture that is the same but for the substitution of the complement in the position noted at the top of the column. Thus, the third column (headed "Axxxxx") shows that substitution of A_1 for A_0 in position 1 yields an ELISA value that is only 1/14.9 as high as the value given. I.e, the reading for the mixture 001001 was 611, but the single-residue complement 101001 had a reading only 1/14.9 as great. (This can be verified by looking for the raw-result reading for the mixture 101001: it is seen that the reading is 41, and 611/41 = 14.9.)

Thus, the presence of A_0 in the first position of the mixture 001001 contributes significantly to the results, since the complement A_1 in that position shows much lower activity. This is likely because a monomer (or monomers) critical for binding is present in binary grouping A_0 but not in A_1. Similarly, A_0 rather than A_1 is preferred in the fifth position. Tolerance to both binary groupings indicates either that a given position will tolerate a wide range of residues, since the side-chains at these positions are not critical for binding, or that both A_0 and A_1 contain select monomers which are suitable replacements for the position. Such tolerance in all sublibraries (i.e. little change with A_0 vs. A_1, B_0 vs. B_1, and C_0 vs. C_1) would suggest the former case.

Figure 8 shows the same type of results for the B sublibrary, with mixture 101101 showing the greatest activity, and Figure 9 shows the results for the C sublibrary, where mixture 110110 shows the greatest activity.

The numerical results of Figures 7-9 (raw results, not the calculated complement comparisons) are graphically depicted in Figures 10-12.

Figure 13 is a graph showing the complement comparisons for each of the top-scoring mixtures of sublibraries A, B and C. The six points on each curve are taken from the six figures across the tops of Figures 7-9, respectively. Note that the higher the y-

coordinate value, the greater the degree to which a given binary grouping (and hence one or more monomers within that binary grouping) is conserved at the respective position.

Table 8 below is a partial listing of the results shown in Figures 7-9:

Table 8: Complement Values for Top-Scoring Mixtures

ELISA Sublibrary	Mixture		ELISA Value	Ratio of ELISA parent to ELISA complement			
	xxxAxx	xxxxAx xxxxxA		Axxxxx	xAxxxx	xxAxxx	
A	001001	611	14.9	5.4	1.2	6.4	14.9
4.5							
	000001	494	8.7	3.1	0.8	6.6	7.6	4.5
	000100	307	1.5	5.1	2.1	1.1	5.0	4.1
	000000	285	1.9	3.3	2.1	0.9	3.4	0.6
	100100	206	0.7	3.3	4.2	1.4	3.0	3.7
B	101101	409	14.1	4.4	2.6	14.1	5.0	7.1
	010110	198	1.6	4.6	2.8	5.1	5.5	4.2
C	110110	268	5.6	3.8	1.4	7.4	8.9	1.4
	111111	233	2.3	2.6	1.3	1.4	3.3	1.2
	111110	194	3.6	2.1	0.7	4.1	3.2	0.8

One way of interpreting these results is to compare the highest-scoring mixtures in each sublibrary, thus allowing a string of motifs to be defined, and hence a string of monomer groupings; this string represents the intersection mixture. The motif for each mixture position is determined, then, and the preferred monomer grouping for each motif is deduced. Intra-sublibrary comparison with single-point complements then indicates the relative importance of each position to the observed binding activity.

Using the above approach, the following results were obtained:

Table 9: Deduced Active Peptide from S1479-1 ELISA Test Results

A(001001)	{		}	D*	Y	A	D*	Y*	A*
B(101101)	⇒	{ cbecbe }	⇒	E	F	G	E	F	G
C(110110)	{		}		hF	P*		hF	P

After this single pass, already the deduced peptide mixture (comprising 2 x 3 x 3 x 2 x 3 x 3 = 324 peptides) is very similar to the known active peptide DVPDYA (the amino acid residues present in the native epitope are marked with asterisks (*) in Table 9). A refinement of this analysis is discussed below relative to Table 10.

The single-point complement analysis indicates that the residues at positions 1, 4 and 5 are highly conserved (*i.e.* significantly reduced activity is shown if any of the residues at those positions are altered). Residue 2 has a "preference" for b (reading the motif 001 in Table 9, with the "c" value taken from Table 3). Residue 3 is not highly conserved, *i.e.* is relatively tolerant to change (note that even the highest complement values are rather low). Residue 6 has a preference for "e" (motif 110), but is tolerant of "d" (motif 111; see the rightmost column at the top entry of library C, showing that a complement substitution leads to very little ELISA value change).

Figure 14 shows a single-point replacement study of the peptide DVPDYA, according to the technique described in the aforementioned Geysen article (*"Strategies for epitope analysis using peptide synthesis"*). It will be seen that the results are quite similar to the above experimental results.

The graphs of Figure 14 show how other amino acids in the positions noted affect the activity of the peptide. In position 1, E is the amino acid that, when substituted at that point, compares best with the activity resulting from D. In position 2, most residues could be acceptable substitutes for V; and in position 3, most residues could be acceptable substitutes for P. In position 4, the native residue D is preferred, with C and H being acceptable replacements; all other substitutions would yield lower activity. Position 5 is seen to be very highly conserved, inasmuch as the ability of the peptide to bind the monoclonal antibody is greatly diminished if Y is substituted by any of the other amino

acids. In position 6, the native residue A is preferred, although G is an acceptable substitute.

As a second pass to the high-score approach (see, *e.g.*, the Library Deconvolution discussion below), in the preferred embodiment a deconvolution procedure is used whereby the experimenter constructs a new library, including each of the possible combinations suggested by a predetermined number of top-scoring mixtures, and/or including each combination involving a mixture having an ELISA score above a certain threshold (such as about 200) -- i.e., only those mixtures that show activity significantly greater than the background result.

Library Deconvolution

The single-pass comparison discussed above ignores the possibility of intersections with other high-activity binary mixtures identified in the preliminary binding study. Each such intersection specifies a different set of peptide mixtures, some of which will probably exhibit significant activity and others of which will exhibit little activity. This can be determined empirically by preparing the peptide mixtures indicated by each possible intersection. In the case of the mixtures listed in Table 8, there are 30 possible inter-sublibrary intersections (30 = 5 x 2 x 3). These are listed in the following table:

Table 10: Deconvolution of Transformed Group Library

(Top 30 Inter-sublibrary intersections for binding with S1479-1)

Intersection (Motif String*)	Intersection mixture (Monomer Grouping String)	ELISA Value	
011-001-110-011-001-110	cbecbe	2652	##
011-001-010-011-001-110	cbXcbe	2827	#
011-001-010-111-001-010	cbXdbX	128	
011-001-010-011-001-010	cbXcbX	1181	#
111-001-010-111-001-010	dbXdbX	106	
001-011-100-011-011-100	bcfccf	68	
001-011-000-011-011-100	bcaccf	99	
001-011-000-111-011-000	bcadca	120	
001-011-000-011-011-000	bcacca	103	
101-011-000-111-011-000	Xcadca	78	

011-001-111-011-001-111	cbdcbd	1573	##
011-001-011-011-001-111	cbccbd	1365	##
011-001-011-111-001-011	cbcdbc	89	
011-001-011-011-001-011	cbccbc	86	
111-001-011-111-001-011	dbcdbc	118	
001-011-101-011-011-101	bcXccX	117	
001-011-001-011-011-101	bcbccX	232	
001-011-001-111-011-001	bcbdcb	1569	##
001-011-001-011-011-001	bcbccb	1257	##
101-011-001-111-011-001	Xcbdcb	1018	#
011-001-111-011-001-110	cbdcbe	2737	##
011-001-011-011-001-110	cbccbe	2457	##
011-001-011-111-001-010	cbcdbX	115	
011-001-011-011-001-010	cbccbX	827	
111-001-011-111-001-010	dbcdbX	117	
001-011-101-011-011-100	bcXccf	87	
001-011-001-011-011-100	bcbccf	104	
001-011-001-111-011-000	bcbdca	123	
001-011-001-011-011-000	bcbcca	98	
101-011-001-111-011-000	Xcbdca	105	

In this table:

(1) * "Motif String" refers to: $A_1B_1C_1$-$A_2B_2C_2$-...-$A_6B_6C_6$;
(2) X = undefined element, 17 amino acid mixtures used in this position;
(3) ## = active intersection, all positions defined; and
(4) # = active position, one or more positions undefined.

This study shows resultant ELISA values for actually tested peptide mixtures, and the results identify cb@cbe (with @ standing for any one of several monomer groupings) as the most strongly binding mixture type, with cb@cb@ and @cb@cb being less strongly binding. The top four binding intersection mixtures have the general form cb@cbe. This includes cbecbe, which is defined by the common intersection of the top activity peptide mixtures from sublibraries A, B and C. Position 3 is tolerant of a monomer or monomers present in monomer groupings c, d and e, and possibly from other groupings. Variation in position 6 from monomer grouping e to d is tolerated, but with loss in binding activity.

Although the general form cb@cbe can be inferred directly from the results in Table 8 (i.e. from the intersections for the top binding binary mixtures from each sublibrary), the data presented in Table 10 unambiguously show that this is the general form of the top binding species.

Tolerance of position 3 to a number of different residues is consistent with the replacement study of Figure 14. The more weakly binding mixture @cb@cb is an example of where a conserved binding pattern has been frame-shifted. (Note that the single-point complement analysis discussed in the foregoing section supports the conclusion that frame-shifting is demonstrated here.)

Accordingly, position 6 can be modified or deleted without complete loss of binding activity. However, the pattern cb@cb must be conserved for any binding to remain. Note that as these peptides are bound to a solid support at the C-terminal, i.e. the RHS (right-hand side), the linkage to the polymer may be acting as a poor substitute for the acceptable position 6 (e) in the non-frame-shifted species.

The user could construct an entire library of all the possibilities suggested by Table 8 and Figure 13, namely all individual peptides suggested by top-activity intersection mixtures. This would lead to the synthesis of 324 (= 2 x 3 x 3 x 2 x 3 x 3) peptides, though, so it would be preferable to undertake another iteration of the method of the invention by creating sublibraries, this time with each sublibrary including a very small number of peptides. In either case, the reduced number of peptides are examined in a second iteration of testing for binding activity.

Table 11 confirms the above results:

D#XDYG	2930		D#XEYP	660	DFXDFA	547
E#XDYA	2916	50	EFXEYG	656	D#XD#P	546
DFXDYG	2875		DYXEYG	652	DFXEFP	544
DYXDYG	2868		D#XEYG	652	DYXE#P	542
DFXDYA	2773	55	EFXEYA	651	EYXEYP	536
D#XDYA	2767		EYXEYA	639	EFXEFA	535
DYXDYA	2624		EYXEFG	639	EFXEYP	529
EFXDYA	1753	60	D#XD#A	639	E#XDFP	523
EYXDYA	1245		D#XD#G	634	EFXDFP	522
E#XDYG	1141	65	DYXE#A	627	EFXDFA	522
DFXD#A	904		EFXE#A	625	DYXEFA	519
DYXEFP	872		DFXEFG	623	EYXEYG	516
DFXD#G	844		DYXDFP	620	EFXDYP	508
DFXEYP	807		EFXEFP	616	DFXE#A	504
DFXE#G	784		DFXDFP	615	EYXDFP	503
EFXEFG	783		D#XEFG	615	E#XEYP	501
DYXEFG	777		EYXD#G	615	EYXD#A	501
D#XDYP	757		D#XDFA	610	EYXEFP	481
DYXDFA	747		DFXEYA	605	E#XE#P	421
D#XEFP	739		EFXDFG	605	E#XEYG	419
DFXDYP	738		DYXEYP	604	EYXDFG	414
DYXEYA	730		DYXD#P	601	E#XDYP	404
DFXEYG	729		EFXE#P	595	E#XD#G	361
EFXE#G	728		D#XDFG	597	EYXE#A	352
D#XE#G	725		DYXE#G	585	E#XE#A	347
DYXDYP	724		D#XE#P	584	E#XE#G	342
EYXDYG	721		D#XE#A	582	E#XD#A	342
D#XEYA	705		EYXDYP	581	E#XEFA	337
DFXE#P	703		D#XDFP	574	E#XD#P	326
DFXEFA	701		DFXDFG	570	E#XDFG	277
EYXEFA	694		EFXDYG	569	E#XDFA	265
EFXD#P	683		EFXD#G	561	E#XEYA	238
DFXD#P	676		D#XEFA	561	EYXE#P	234
EYXD#P	672		EFXD#A	558	EYXE#G	215
DYXDFG	672		DYXD#A	554	E#XEFG	204
DYXD#G	664		EYXDFA	551	E#XEFP	197

Table 11 presents rank-ordered ELISA results for the binding of 108 mixtures derived from the top intersection mixtures cbccbe, cbdcbe, and cbecbe to S1479-1. Five positions were defined, *i.e.* single amino acids coupled. Position 3, which appears to be highly replaceable, was coupled as a mixture of all components of c, d and e, namely: A, G, P, D, E, S, N and Q (defined here as X). HomoPhe is defined in Table 11 as #. These were compared with four copies each of DVPDYA (epitope, positive control) and ASQGGL (an unrelated peptide unlikely to bind to S1479-1, negative control).

The experiment reflected in Table 11 clearly identifies seven strongly binding mixtures with five defined residue positions. Many strongly resemble the known epitope, with positions 4 and 5 (-DV-) being conserved throughout. Further deconvolution by defining X would then identify a set of peptides which bind strongly to S1479-1. Strongly binding peptides of the form E#XDYA, for example, differ significantly (*i.e.* in at least two positions) from the known epitope and could be considered as mimotopes or analogs.

Note that the replacement study illustrated in Figure 14 was performed using single peptides, rather than mixtures. Consequently, it may be expected that different patterns could emerge vis-a-vis the complement study of Figure 13 and Table 8, since in the latter multiple replacements are explored simultaneously.

In the method of the invention, data are not excluded until late in the iteration process, whereas in the iterative approach (such as that described by Geysen), data are excluded at each iterative step. For example, in a library of the form DDXXXX (where D is a defined position and X is a mixed position), the top activity mixture D'D"XXXX is carried through the iteration D'D"DXXX. The top activity mixture is then carried through the next iteration step, and so on. Hence, mixtures that may have high but not the highest binding are excluded in the process, even though some of these may yield useful target species after a number of iterations. The present method overcomes this loss of important data by the simultaneous exploration of multiple possibly active oligomers.

Least Regression Analysis

A conventional linear regression analysis can be carried out on the results shown in Figures 7-9, by generating 192 simultaneous equations, with each monomer grouping being assigned a partial activity coefficient based upon its ELISA value. Applicants have done this, and have found the results to be quite similar to those achieved above.

Other methods are suitable for analyzing the results.

Matrix Operations to Generate Paired Groups

An even number of monomer groupings can be divided into two paired sets in a variety of ways. The organization process can be described by two matrix operations. The first mixes the monomer groupings, and the second selects either group from the monomer grouping set. The following example constructs binary grouping B(0) from the set of monomer groupings given above.

In the simple case given here, bisection by observation is sufficient. However, examination of symmetry functions may be necessary when more complex libraries are constructed.

Table 12: Matrices to Generate Sublibraries

Matrix to Select Group 0	Matrix to Generate Sublibrary B			B(0)
1 0 0 0 0 0	0 0 0 0 0 1	a		f
0 1 0 0 0 0	1 0 0 0 0 0	b		a
0 0 1 0 0 0	0 1 0 0 0 0	c	=	b
0 0 0 0 0 0	0 0 1 0 0 0	d		0
0 0 0 0 0 0	0 0 0 1 0 0	e		0
0 0 0 0 0 0	0 0 0 0 1 0	f		0

Conclusion

In the method described here, potentially useful leads are not excluded at an early stage of deconvolution. Rather, general features of all residue positions are established, in particular (1) the preferred monomer groupings at all positions, and (2) the general tolerance to variation at each position. Furthermore, more than one general binding form can be deconvoluted simultaneously by the method. This can be useful in identifying two unrelated, but potent, binding patterns.

CLAIMS:

1. A method for determining which oligomer of a plurality of synthesized oligomers contributes highly to a known activity with respect to a target, including the steps of:

(1) preparing predetermined groupings of preselected monomers;

(2) from the predetermined monomer groupings, preparing predetermined first and second binary groupings of monomer groupings;

(3) forming a plurality of first oligomer mixtures from the first and second binary groupings, each said first oligomer mixture being formed by concatenation of N of the first and second binary groupings onto a first support structure, where N is a number representing a predetermined oligomer length, thereby generating a first plurality of synthesized oligomers of length N with each position in the first plurality of synthesized oligomers occupied by one of said preselected monomers;

(4) from the predetermined monomer groupings, preparing predetermined third and fourth binary groupings of monomer groupings;

(5) forming a plurality of second oligomer mixtures from the third and fourth binary groupings, each said second oligomer mixture being formed by concatenation of N of the third and fourth binary groupings onto a second support structure, thereby generating a second plurality of synthesized oligomers of length N with each position in the second plurality of synthesized oligomers occupied by one of said preselected monomers;

(6) from the predetermined monomer groupings, preparing predetermined fifth and sixth binary groupings of monomer groupings;

(7) forming a plurality of third oligomer mixtures from the fifth and sixth binary groupings, each said third oligomer mixture being formed by concatenation of N of the fifth and sixth binary groupings onto a third support structure, thereby generating a third plurality of synthesized oligomers of length N with each position in the third plurality of synthesized oligomers occupied by one of said preselected monomers;

(8) reacting the first, second and third pluralities of oligomer mixtures with the target;

(9) determining a subset of oligomer mixtures of said first, second and third pluralities of oligomer mixtures, respectively, which contribute highly to activity with respect to the target;

(10) determining at least one intersection mixture of the oligomer mixtures determined in step 9.

2. The method of claim 1, further including the steps of:

synthesizing at least one oligomer represented in said intersection mixture;

reacting said at least one synthesized oligomer with the target; and

determining an amount of activity of said at least one active oligomer in reaction with the target.

3. The method of claim 1, wherein the monomers in at least one said monomer grouping are selected to have similar chemical properties.

4. The method of claim 1, wherein said first and second binary groupings are selected to include mutually exclusive sets of monomer groupings.

5. The method of claim 1, wherein step 10 is carried out by:

determining which monomer grouping is represented at each of N positions in a predetermined number of oligomer mixtures selected from said subset of oligomer mixtures.

6. The method of claim 5, wherein at least one of said predetermined number of oligomer mixtures is selected on the basis of contributing most highly to activity with respect to the target.

7. A method of elucidating which of a plurality of oligomers is responsible for a known reaction, including the steps of:

(1) selecting a plurality of monomers, the monomers being selected as being potential contributors to the known activity;

(2) preparing a plurality of monomer groupings from the selected monomers, each monomer grouping including at least one of the selected monomers;

(3) forming a plurality of pairs of binary groupings of the monomer groupings, where each binary grouping pair comprises a first set of said monomer groupings and a second set of said monomer groupings, said first and second sets including mutually exclusive subsets of said monomer groupings;

(4) generating a sublibrary of oligomer mixtures from each binary grouping pair by synthesizing a plurality of oligomers on a support structure by coupling together different sequences of each binary grouping from each said pair of binary groupings;

(5) reacting each of the synthesized oligomer mixtures in each sublibrary in the known activity;

(6) determining which of the synthesized oligomer mixtures in each sublibrary are highly active in the known reaction; and

(7) cross-correlating the determinations according to step 6 to elucidate a subset of the oligomer mixtures, the subset including oligomers that are highly active in the known reaction.

8. The method of claim 7, wherein the number of sublibraries formed in step 3 is three.

9. The method of claim 8, wherein the monomer groupings are prepared in step 2 to include monomers having similar chemical properties.

10. The method of claim 7,further comprising the additional step of testing the subset of elucidated oligomers in the known reaction.

11. A set of oligomer mixtures prepared according to claim 1.

12. The set of claim 11, wherein the oligomers are oligopeptides.

13. The set of claim 12, wherein the oligopeptides are hexapeptides.

14. The set of claim 11, wherein the oligomers are oligonucleotides.

15. The set of claim 11, wherein the oligomers are oligopeptoids.

16. The set of claim 15, wherein the oligopeptoids are tripeptoids.

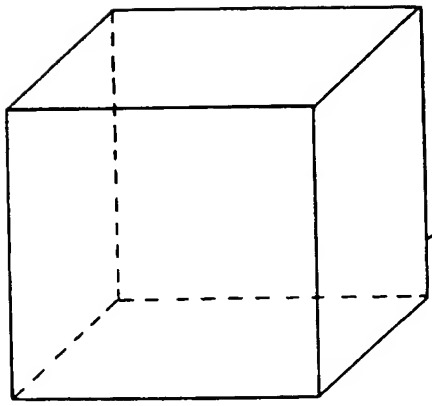


FIG.1

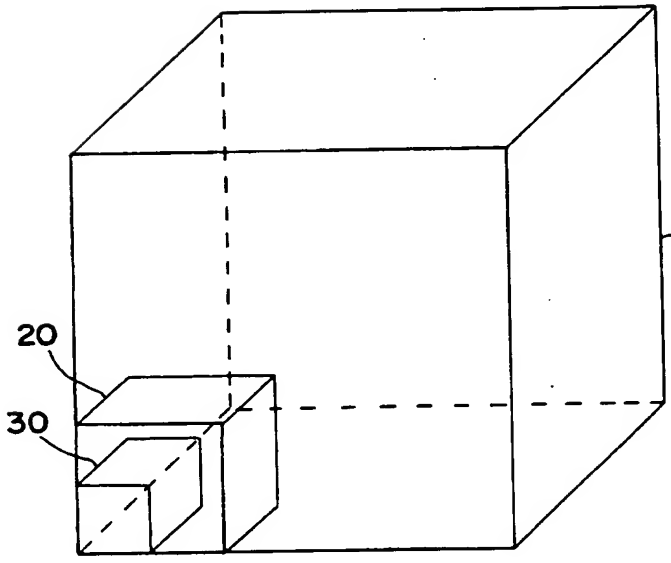


FIG.2

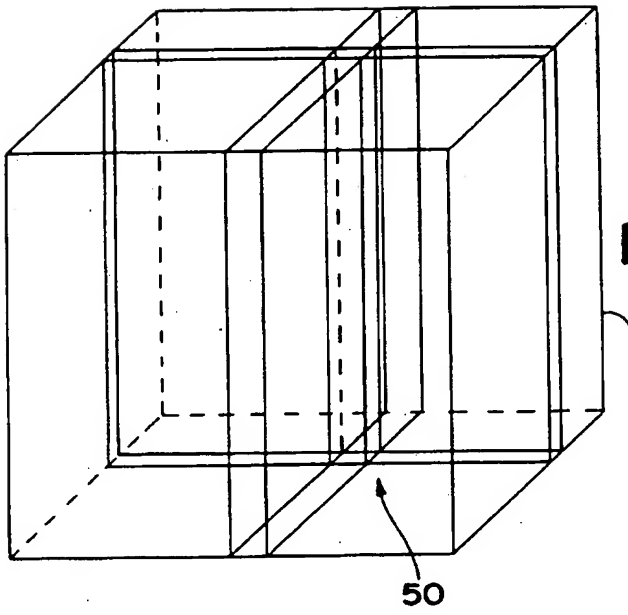


FIG. 3

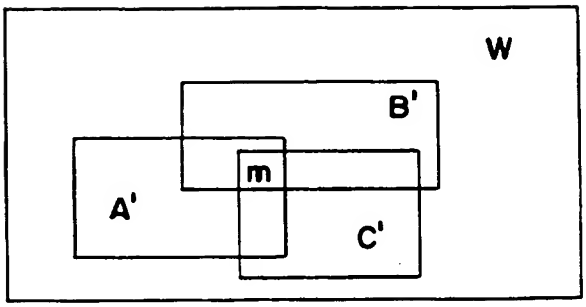


FIG.4

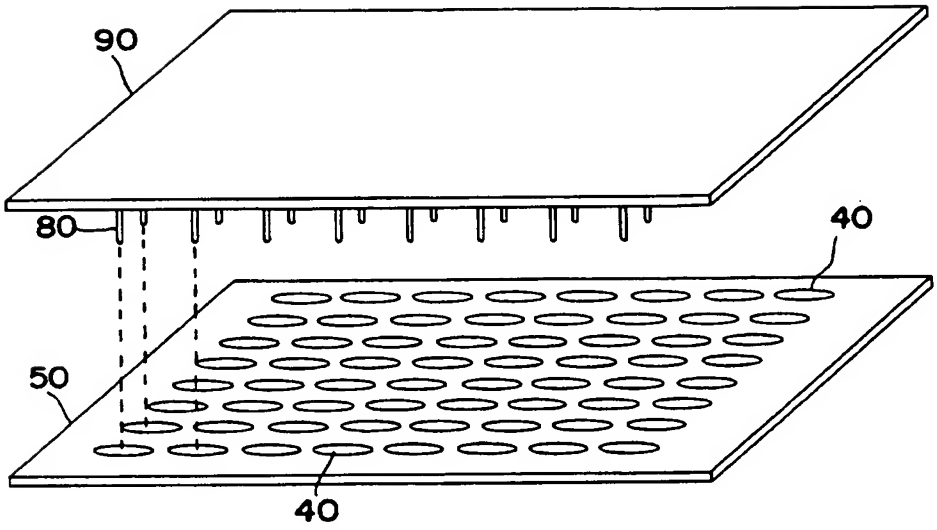


FIG.5

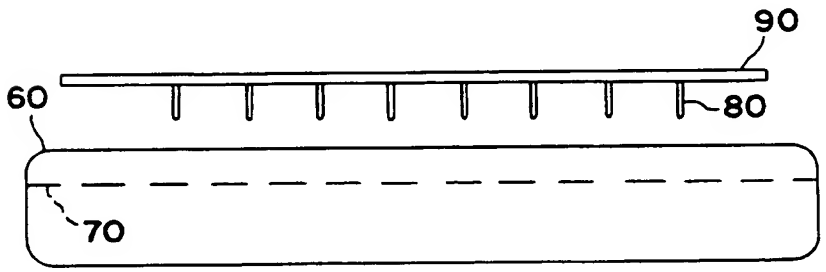


FIG.6

Group 1 & 2	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
611 001001	14.9	5.4	1.2	6.4	14.9	4.5
494 000001	8.7	3.1	0.8	6.6	7.6	1.7
307 000100	1.5	5.1	2.1	1.1	5.0	4.1
285 000000	1.9	3.3	2.1	0.9	3.4	0.6
206 100100	0.7	3.3	4.2	1.4	3.0	3.7
158 010001	2.3	0.3	1.4	3.4	3.6	1.8
149 001100	3.0	2.5	0.5	1.1	3.2	1.6
147 100000	0.5	1.9	1.7	0.7	2.0	2.6
135 001000	1.6	1.6	0.5	0.9	2.4	0.2
113 011001	4.2	0.2	0.7	3.8	4.3	1.4
95 001101	2.6	3.2	1.3	0.2	3.1	0.6
86 010000	1.1	0.3	1.0	1.4	1.4	0.5
85 000010	1.2	1.4	1.5	1.4	0.3	1.3
85 101000	0.6	1.8	0.6	1.7	1.7	2.1
82 011000	1.8	0.6	1.0	1.4	1.5	0.7
76 110000	0.9	0.5	1.7	1.2	1.9	1.1
75 000101	1.3	1.6	0.8	0.2	1.6	0.2
73 100010	0.9	1.8	1.5	1.1	0.5	2.4
69 100110	1.1	2.5	2.4	0.9	0.3	2.6
69 010110	2.5	1.1	2.2	1.1	1.2	2.1
68 110001	0.4	1.2	2.5	2.1	1.7	0.9
65 000011	2.1	1.5	1.6	1.4	0.1	0.8
63 110100	1.1	0.3	2.9	0.8	2.3	2.0
62 000110	0.9	0.9	1.3	0.7	0.2	1.3
61 010010	1.5	0.7	1.1	0.9	0.7	1.4
60 010100	1.0	0.2	1.0	0.7	0.9	1.3

FIG. 7A

Group 1 & 2	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
59 011100	2.7	0.4	1.0	0.7	1.9	2.0
57 100001	0.1	0.8	1.4	1.0	1.8	0.4
57 001010	1.1	1.1	0.7	1.2	0.4	1.4
56 100101	0.7	1.8	1.5	1.0	2.1	0.3
54 011010	1.8	0.9	0.9	1.7	0.7	2.1
50 101010	0.9	1.7	0.7	1.7	0.6	1.9
49 101100	0.3	2.2	0.2	0.6	1.7	1.3
47 001110	1.6	1.5	0.8	0.8	0.3	1.5
47 000111	1.7	1.4	1.5	0.7	0.6	0.8
46 111000	0.6	0.5	0.6	2.1	1.5	1.7
46 010101	1.4	0.6	1.5	0.3	1.4	0.8
44 010011	1.1	0.7	1.7	1.3	0.3	0.7
41 101001	0.1	1.5	0.7	1.1	1.5	0.5
41 001011	1.5	1.6	0.6	1.3	0.1	0.7
40 110010	0.7	0.5	1.3	1.4	0.5	1.0
39 110011	0.9	1.3	2.2	1.8	0.6	1.0
37 101101	0.4	2.1	0.7	0.9	2.2	0.8
33 010111	1.5	0.7	1.7	0.8	0.7	0.5
32 110101	0.7	0.6	1.8	0.5	1.5	0.5
31 001111	1.8	1.6	0.7	0.8	0.3	0.7
31 011110	1.5	0.7	0.4	0.6	0.5	1.6
31 100011	0.5	0.8	1.1	1.1	0.5	0.4
30 111010	0.6	0.6	0.8	1.4	0.7	1.7
30 011101	1.7	0.3	0.7	0.3	1.5	0.5
29 101110	0.6	1.4	0.4	0.6	0.6	1.7
28 110110	0.4	0.4	1.3	0.7	0.4	1.3

FIG. 7B

Group 1 & 2	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
27 101011	0.7	1.5	0.9	1.6	0.7	0.5
27 111001	0.2	0.7	0.4	1.5	1.5	0.6
27 100111	0.6	1.2	1.6	0.9	0.5	0.4
26 011011	1.4	0.6	0.6	1.3	0.2	0.5
22 111100	0.4	0.4	0.3	0.5	1.0	1.2
22 110111	0.7	0.8	1.0	0.6	0.7	0.8
21 111111	1.1	1.2	1.0	1.2	1.2	1.0
21 111110	0.7	0.7	0.8	0.7	1.0	1.0
20 011111	1.0	0.6	0.6	0.8	0.7	0.6
18 111101	0.6	0.5	0.6	0.7	0.9	0.8
18 111011	0.7	0.7	0.5	0.9	0.7	0.6
17 101111	0.5	0.8	0.6	0.6	0.5	0.6

FIG.7C

Group 3 & 4	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
409 101101	14.1	4.4	2.6	14.1	5.0	7.1
198 010110	1.6	4.6	2.8	5.1	5.5	4.2
159 100101	3.5	2.4	0.4	2.5	3.1	2.2
126 100000	1.0	2.6	2.4	1.8	3.7	2.0
126 000000	1.0	1.0	1.4	1.9	2.2	1.5
125 010000	2.6	1.0	2.3	3.5	3.2	1.2
125 110110	0.6	4.2	1.7	2.3	3.5	1.9
102 010001	3.3	1.2	1.8	3.5	3.5	0.8
93 111101	2.0	0.2	1.4	1.8	1.3	1.8
89 001000	1.7	1.6	0.7	2.1	1.5	1.6
86 000001	1.4	0.8	1.6	1.9	1.8	0.7
81 101111	1.7	1.1	1.6	1.7	0.2	1.4
75 111110	1.1	1.3	0.6	1.1	1.4	1.0
72 111111	1.1	0.9	1.1	1.1	0.8	1.0
72 100100	1.1	2.0	1.2	0.6	2.4	0.5
71 001011	1.5	1.1	1.4	1.4	1.3	1.2
70 011110	0.9	1.4	0.4	1.7	2.1	1.1
69 111010	1.7	1.6	1.3	0.9	2.2	1.0
67 111011	1.1	1.4	1.3	0.9	1.3	1.0
66 110111	1.4	1.3	0.9	1.3	1.0	0.5
66 110101	2.3	0.4	0.7	2.1	1.0	1.8
66 100011	1.3	1.3	1.4	1.3	1.0	1.9
65 011111	0.9	1.3	1.4	1.0	1.4	0.9
65 000100	0.9	1.8	1.5	0.5	1.5	1.4
63 011011	0.9	0.9	2.2	1.0	1.1	1.5
63 100001	0.7	2.0	2.2	0.4	1.0	0.5
60 001010	1.4	1.5	1.1	1.2	0.7	0.8

FIG. 8A

Group 3 & 4	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
59 101110	1.2	0.8	2.0	1.4	1.0	0.7
58 011001	1.1	1.1	0.6	1.2	0.9	1.1
58 101100	1.3	1.1	0.8	1.1	1.0	0.1
57 000010	1.7	1.5	1.0	1.3	0.5	1.2
55 001001	1.9	0.9	0.6	1.9	0.8	0.6
54 110010	1.4	1.6	0.8	0.4	1.1	1.1
54 011000	1.7	0.6	0.4	1.6	1.3	0.9
53 101000	0.6	1.7	0.4	0.9	1.2	1.8
53 111001	0.9	1.8	1.7	0.6	0.8	1.7
52 111100	1.5	0.9	1.4	1.7	0.7	0.6
51 100111	1.9	0.8	0.6	0.8	0.3	1.7
50 110011	1.7	0.8	0.7	0.8	1.6	0.9
50 001110	0.8	0.7	1.2	0.8	1.2	1.0
49 000011	0.7	1.7	0.7	1.8	0.6	0.9
49 001111	0.6	0.8	1.8	0.7	1.7	1.0
48 110000	0.4	0.4	1.5	1.3	0.9	1.5
47 101011	0.7	0.7	0.7	0.6	1.6	1.1
47 010111	0.7	1.7	0.7	1.6	1.6	0.2
47 011101	0.5	1.6	1.6	0.8	0.7	1.4
46 000101	0.3	1.6	1.6	0.5	1.7	0.7
43 000110	1.4	0.2	0.9	0.8	0.7	1.6
43 101010	0.7	0.6	1.3	0.7	0.8	0.9
43 001100	0.7	1.3	0.7	0.5	0.9	1.5
41 011010	0.6	0.7	1.1	0.6	0.8	0.7
39 010010	0.7	0.7	1.0	0.2	0.3	1.3
36 010100	1.0	0.6	1.1	0.3	0.2	1.2
36 110100	1.0	0.5	0.7	0.8	0.3	0.5

FIG.8B

Group 3 & 4	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
34 100010	0.6	0.6	0.8	1.1	0.3	0.5
34 011100	0.7	0.8	0.9	0.6	0.5	0.7
31 110001	0.3	0.5	0.6	0.5	0.6	0.6
31 111000	0.6	0.6	0.6	0.6	0.4	0.6
30 100110	0.7	0.2	0.5	0.9	0.4	0.6
29 101001	0.5	0.5	0.5	0.1	0.6	0.5
29 001101	0.1	0.6	0.6	0.5	0.6	0.7
29 010011	0.6	0.6	0.5	0.6	0.3	0.7
29 010101	0.4	0.6	0.6	0.3	0.6	0.8
27 000111	0.5	0.6	0.6	0.6	0.6	0.6

FIG. 8C

Group 5 & 6	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
268 110110	5.6	3.8	1.4	7.4	8.9	1.4
233 111111	2.3	2.6	1.3	1.4	3.3	1.2
194 111110	3.6	2.1	0.7	4.1	3.2	0.8
185 110111	2.4	3.4	0.8	2.2	3.9	0.7
166 111011	2.2	3.0	2.0	0.7	3.0	3.5
101 011111	0.4	2.2	1.3	1.3	2.1	1.9
93 101110	2.4	0.5	1.3	1.7	2.2	1.1
88 101111	2.0	0 4	1.6	1.6	1.2	0.9
83 110011	2.2	1.7	0.5	0.4	1.5	2.3
78 010111	0.4	2.9	0.8	2.1	2.8	1.6
77 011011	0.5	2.5	2.0	0.8	2.6	2.1
71 101101	1.6	1.0	1.2	1.9	0.8	1.7
71 100110	3.0	0.3	0.8	2.8	2.6	1.3
70 111101	1.5	1.0	1.5	1.3	0.3	1.1
61 111100	1.7	1.5	2.0	2.3	0.3	0.9
57 100101	2.9	1.2	0.8	1.4	1.0	2.1
56 101011	1.8	0.3	1.2	0.6	1.5	1.0
56 101010	2.2	1.2	2.2	0.6	1.6	1.0
56 110001	2.1	1.4	1.0	1.2	0.7	2.4
55 100111	2.0	0.3	0.6	1.1	1.0	0.8
55 111001	1.8	1.5	1.0	0.8	0.3	2.0
54 011110	0.3	1.4	1.1	1.5	1.5	0.5
48 011101	0.7	1.1	1.7	1.6	0.5	1.4
48 110101	1.7	0.8	0.7	0.9	0.3	1.6
48 100011	2.1	0.6	0.9	0.9	1.2	1.9
48 010110	0.2	2.0	0.9	1.8	2.2	0.6
47 111010	1.3	0.8	1.3	0.2	1.7	0.3

FIG. 9A

Group 5 & 6	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
45 001101	0.6	0.9	2.3	1.7	1.0	1.9
45 001111	0.5	0.4	1.7	1.5	1.0	1.2
42 101100	1.8	0.7	1.6	1.2	0.5	0.6
40 100001	1.6	0.7	1.1	0.7	0.8	1.6
38 010011	0.5	1.7	0.5	0.5	1.4	1.5
38 001110	0.4	0.7	1.6	1.6	1.6	0.8
37 011010	0.8	1.4	1.4	0.7	1.2	0.5
37 101001	1.4	0.7	0.9	0.5	0.7	1.0
36 110010	1.4	1.4	0.8	0.1	1.6	0.4
36 101000	1.6	1.3	1.4	0.9	0.6	1.0
35 011100	0.6	1.5	1.6	1.1	0.6	0.7
31 011000	1.1	1.3	1.6	0.9	0.8	1.0
31 001011	0.6	0.4	1.3	0.7	1.2	1.2
30 011001	0.5	1.2	1.1	0.6	0.4	1.0
30 110100	1.4	1.1	0.5	1.3	0.1	0.6
28 010101	0.6	1.4	0.6	1.0	0.4	1.3
27 100100	1.4	0.9	0.6	1.1	0.4	0.5
27 000111	0.5	0.3	0.6	1.2	1.4	1.1
27 010001	0.5	1.1	0.9	1.0	0.7	1.4
27 111000	0.9	0.8	1.2	0.4	0.6	0.5
26 001001	0.7	0.9	1.0	0.6	0.8	1.1
26 010010	0.7	1.2	0.7	0.5	1.4	0.7
26 001010	0.5	0.7	1.2	0.7	1.1	0.8
25 000001	0.6	0.9	1.0	1.3	1.1	1.1
25 100000	1.1	1.1	0.7	0.9	1.0	0.6
25 100010	1.2	0.7	0.4	0.4	1.0	0.5

FIG. 9B

Group 5 & 6	Axxxxx	xAxxxx	xxAxxx	xxxAxx	xxxxAx	xxxxxA
24 000110	0.3	0.5	0.6	1.1	1.2	0.9
24 001100	0.6	0.7	1.2	1.0	0.6	0.5
23 001000	0.6	0.7	1.0	1.0	0.9	0.9
23 110000	1.2	0.9	0.9	0.8	0.6	0.4
23 000000	0.9	1.2	1.0	1.2	1.1	0.9
23 000011	0.5	0.6	0.7	0.9	0.9	1.1
22 010100	0.7	1.1	0.6	1.2	0.5	0.8
21 000010	0.8	0.8	0.8	0.9	0.9	0.9
20 000100	0.7	0.9	0.8	0.9	0.8	1.0
20 000101	0.4	0.7	0.4	0.8	0.7	1.0
19 010000	0.8	0.8	0.6	0.9	0.7	0.7

FIG.9C

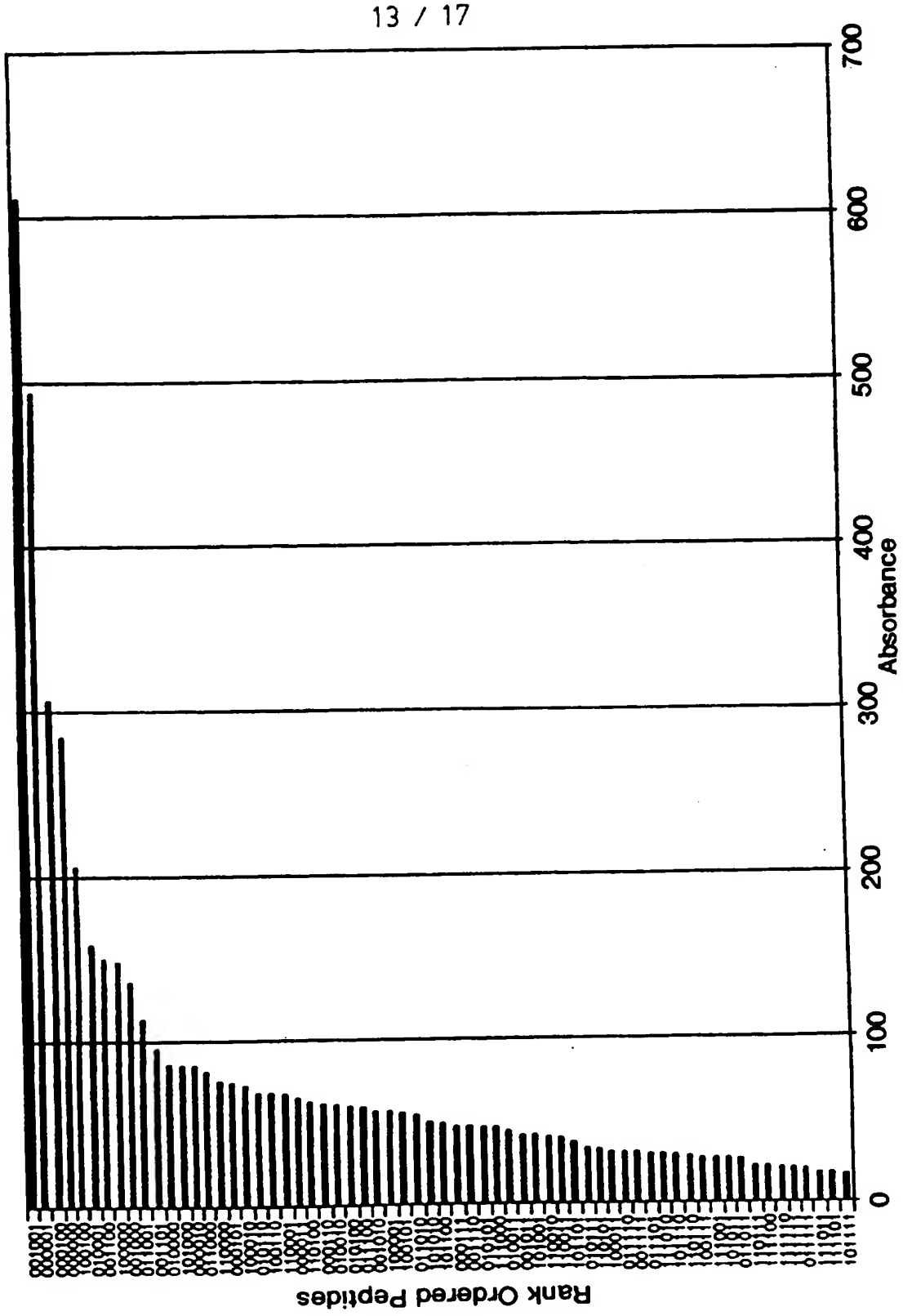
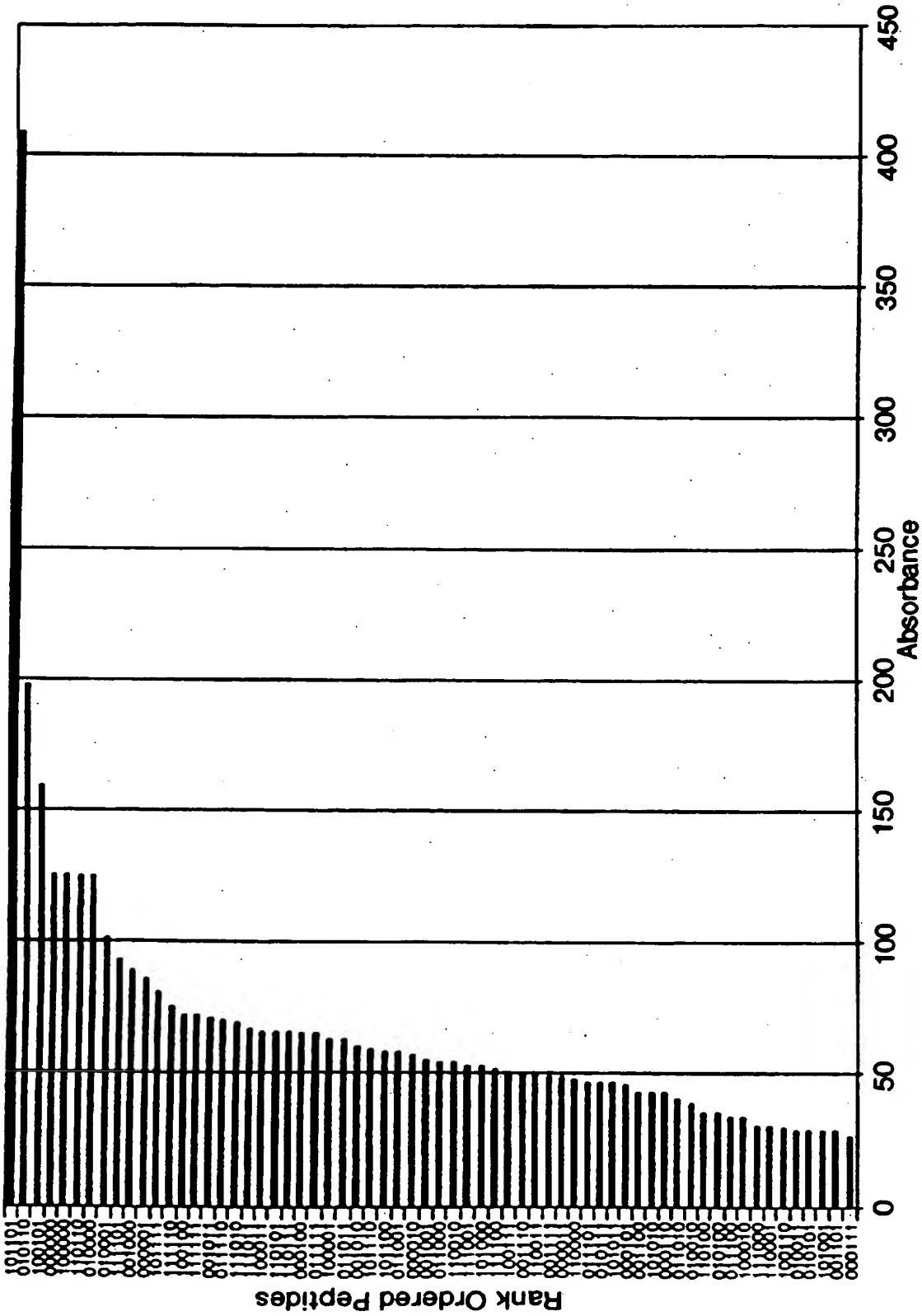

700
600
500
400
300
200
100
0
Absorbance
Rank Ordered Peptides

FIG. 10


450
400
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300
250
200
150
100
50
0
Absorbance
Rank Ordered Peptides

FIG. 11

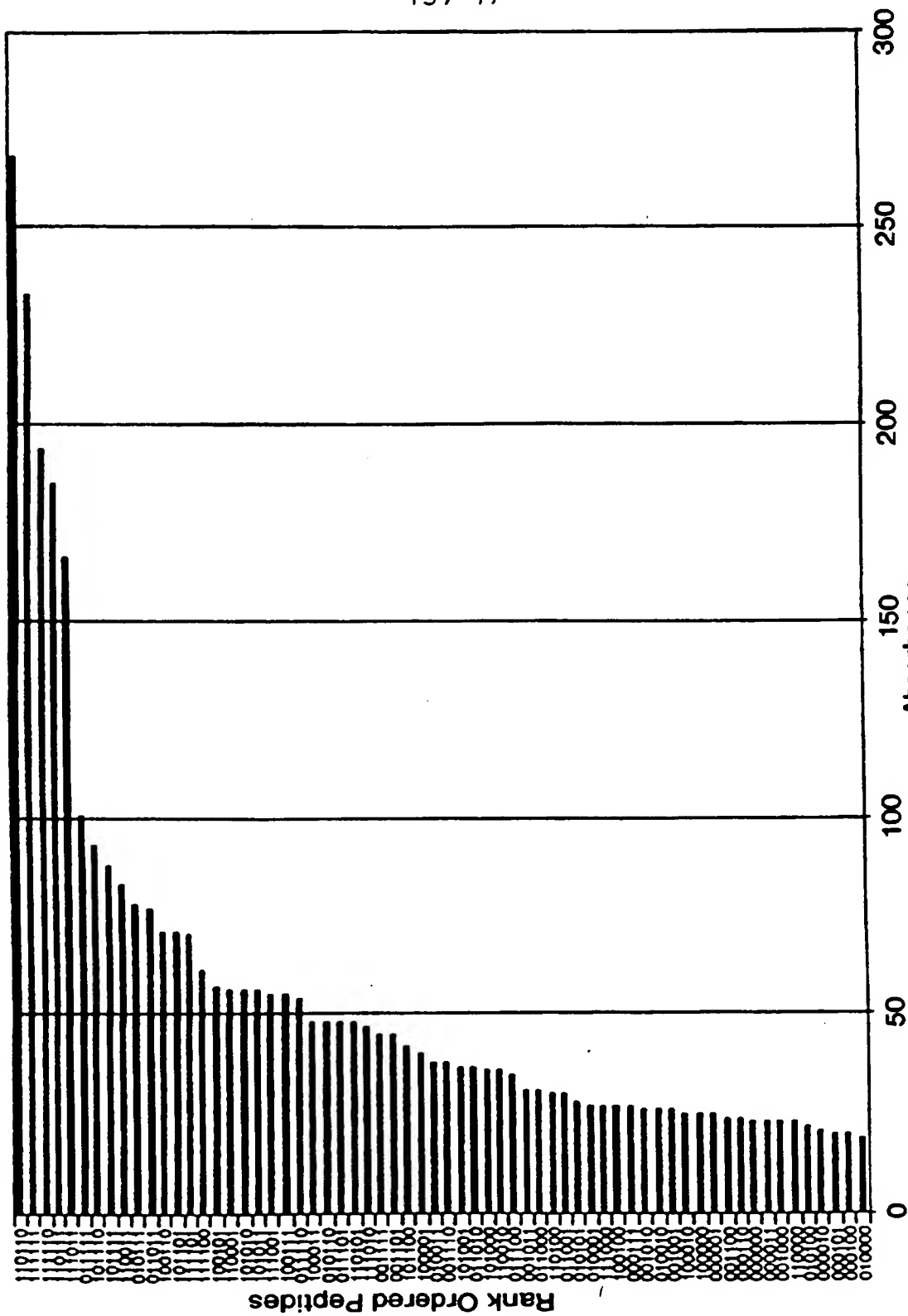

FIG. 12

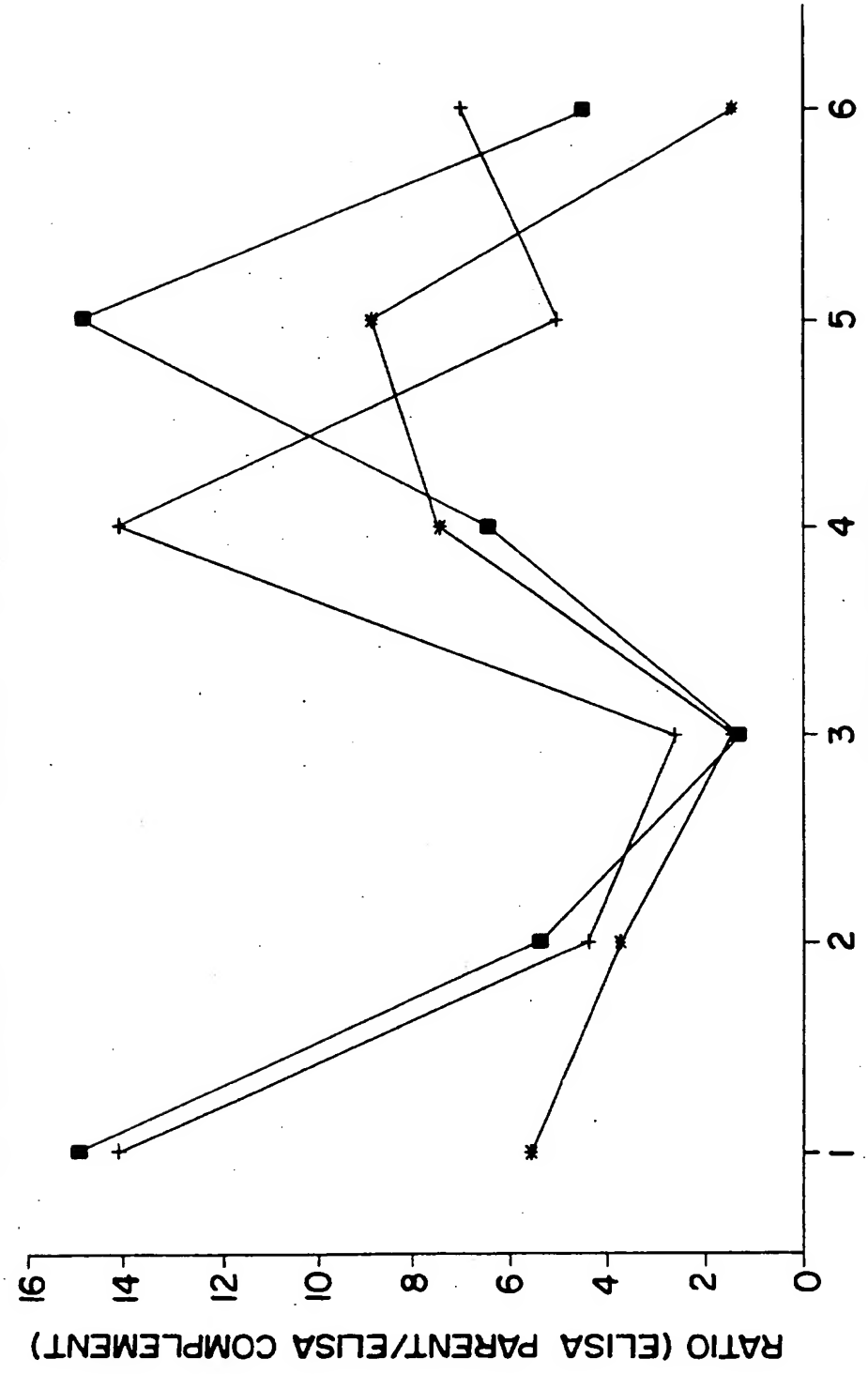
A(001001)
B(101101)
C(110110)
RATIO (ELISA PARENT/ELISA COMPLEMENT)
0
2
4
6
8
10
12
14
16
1
2
3
4
5
6
RESIDUE POSITION

FIG.13

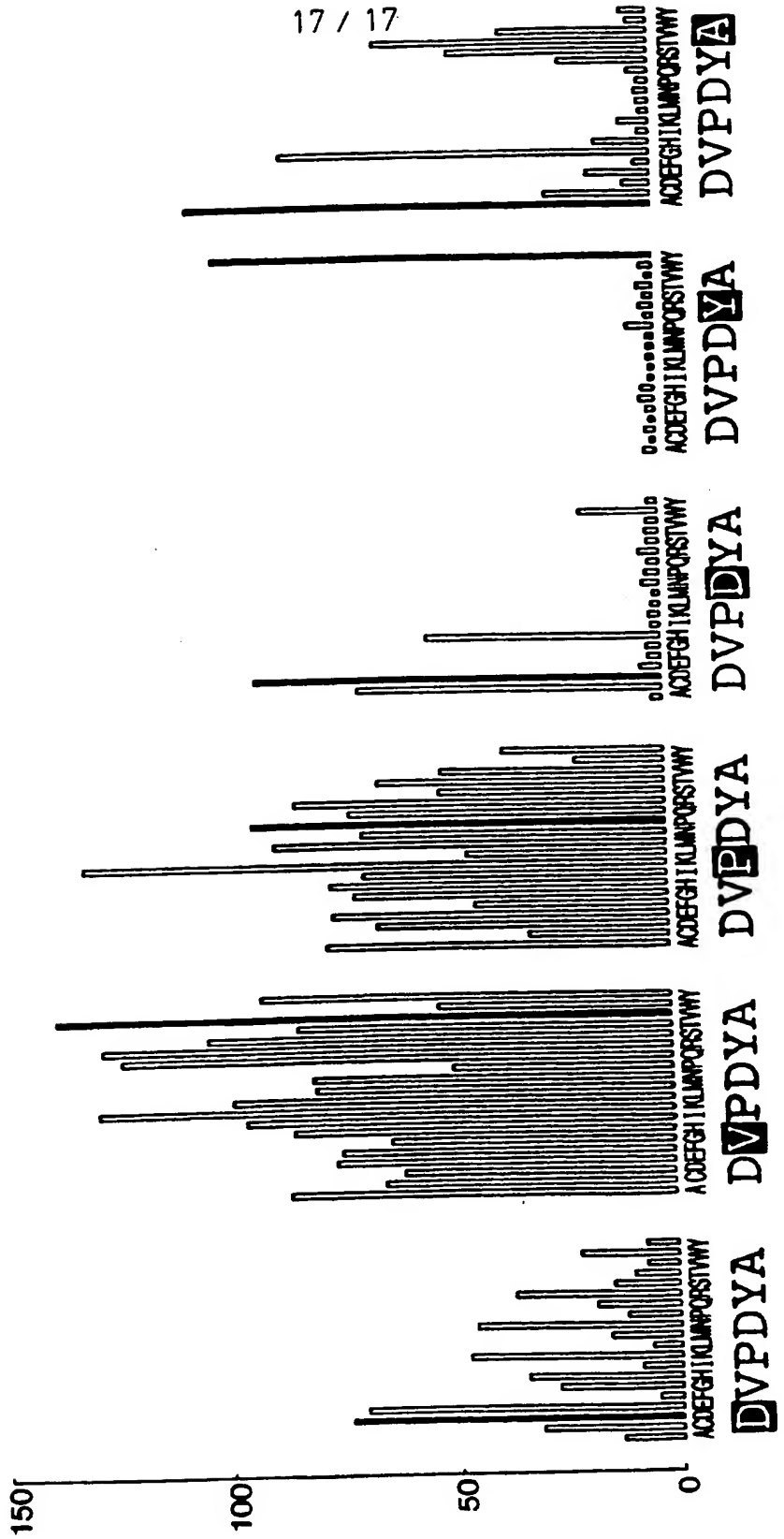
150
100
50
0
ACDEFGHIKLMNPQRSTVWY
DVPDYA
DVPDYA
DVPDYA
DVPDYA
DVPDYA
DVPDYA

FIG.14

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00647

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: G01N 33/53, 33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: G01N - KEYWORDS [MIMOTOPE, PARATOPE, SELECTIDE, EPITOPE, ANTIGEN, LIGAND RECEPTOR, ENZYME, DETERMINANT]

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
File ORBIT
File CAS Online
[MIMOTOPE, PARATOPE, SELECTIDE, EPITOPE, ANTIGEN, LIGAND RECEPTOR, ENZYME DETERMINANT]

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Journal of the American Chemical Society, Vol. 117, No. 19 Issued 17 May 1995. Benoît Déprez et al. "Orthogonal Combinatorial Chemical Libraries", pp. 5405-5406.	1-16
X	Nature, Vol. 251, Issued 15 February 1991, S.P.A. Fodor et al. "Light-directed, Spatially Addressable Parallel Chemical Synthesis", pp.767-773.	1-16
X	AU,A, 56478/86 (COMMONWEALTH SERUM LABORATORIES COMMISSION) 30 October 1986, Page 7, Line 5 to Page 14 Line 27, Page 15 Line 12 to Page 26 Line 22.	1-16

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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Date of the actual completion of the international search
17 January 1996

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00647

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

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A	Nature, vol. 354, Issued 7 November 1991, R.A. Houghten et al "Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery", pp. 84-86	1-16
A	Science, vol. 249, Issued 27 July 1990, J.K. Scott et al "Searching for Peptide Ligands with an Epitope Library", pp. 386-390.	1-16
A	Peptide Research, vol. 5, No. 5, Issued 1992, A. van Amerongen et al. "Peptides Reactive with a Transmission-Blocking Monoclonal Antibody Against Plasmodium falciparum Pfs 25: 2000 - fold Affinity Increase by PEPSCAN-Based Amino Acid Substitutions", pp. 269-274.	1-16
A	Bioorganic & Medicinal Chemistry Letters, Vol. 3, No. 3, Issued 1993, J.M. Kerr et al. Identification of Antibody Mimotopes Containing Non-Natural Amino Acids by Recombinant and Synthetic Peptide Library Affinity Selection Methods", pp. 463-468.	1-16
A	Peptide Research, Vol. 7, No. 1, Issued 1994, A. Wallace et al. "A Multimeric Synthetic Peptide Combinatorial Library", pp. 27-31.	1-16
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INTERNATIONAL SEARCH REPORT

International Application No.
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This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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END OF ANNEX